50th ANNIVERSARY OF THE FOUNDING OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE

VOLUME 2 September 1953 NUMBER 5

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A

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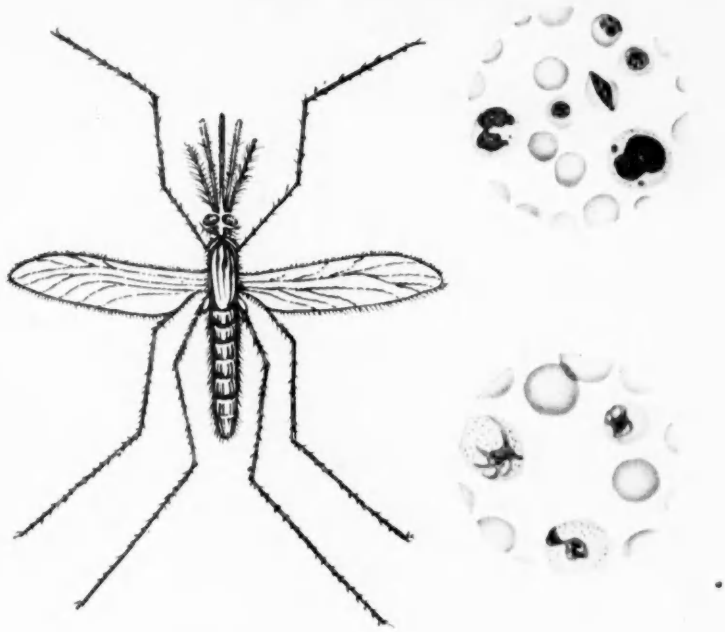

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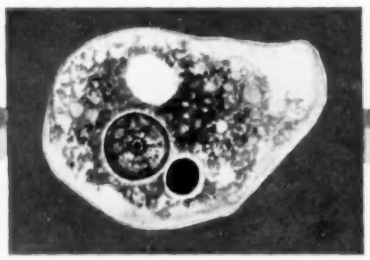

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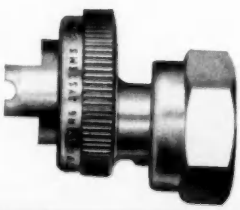

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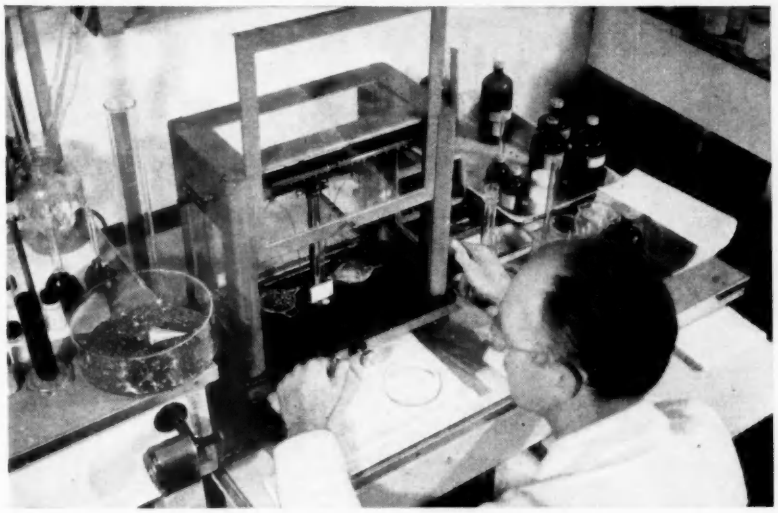

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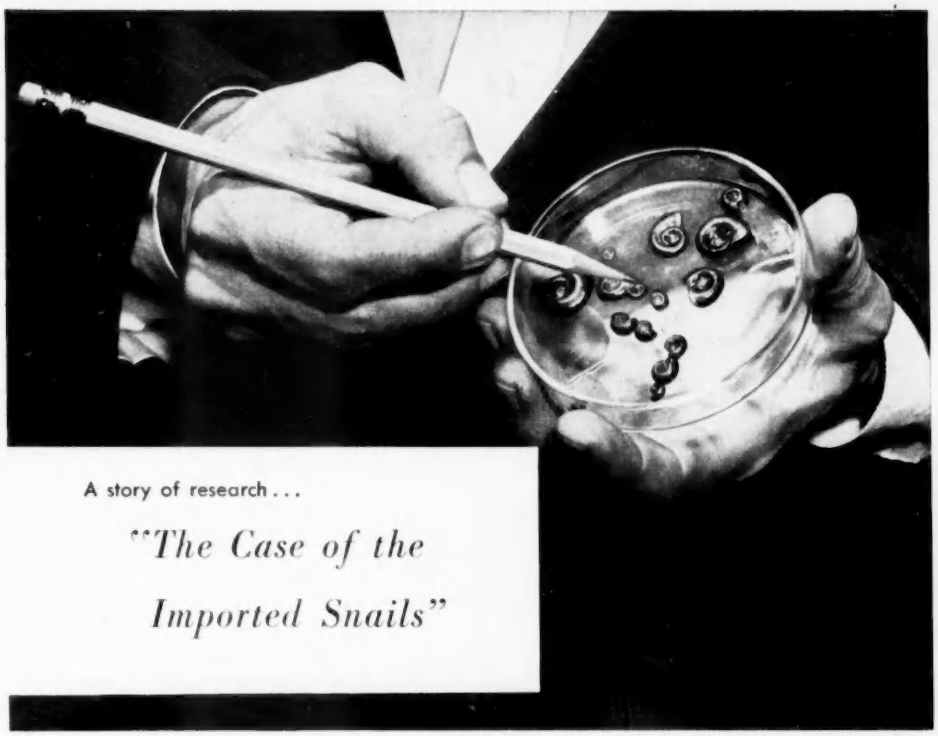

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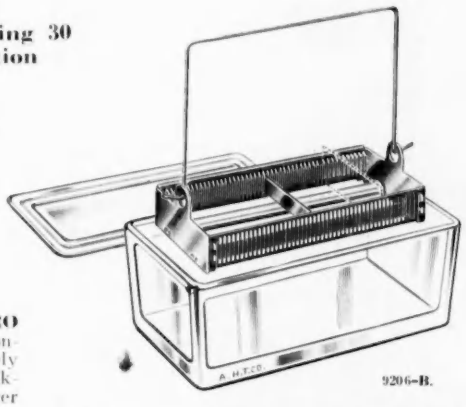

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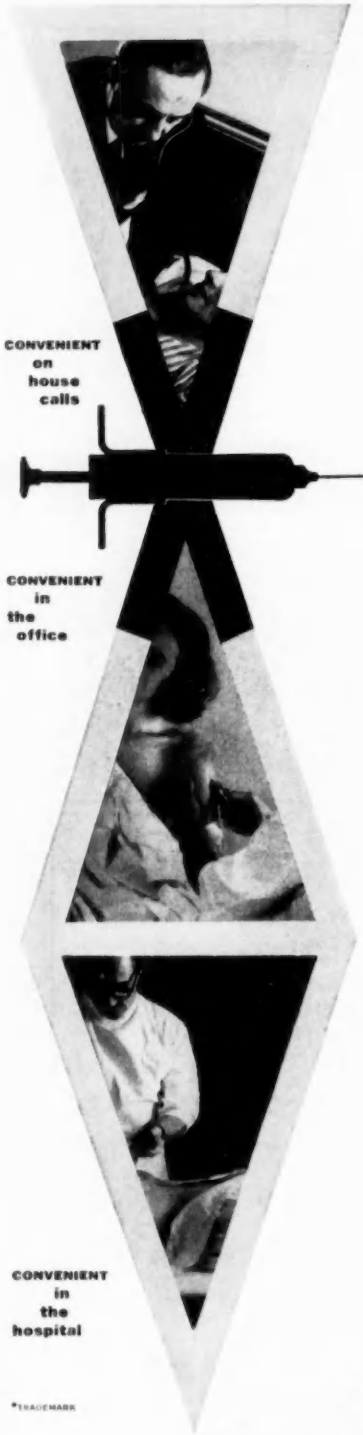

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VECTORS AND RESERVOIRS OF VIRUS DISEASES[1]

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The present century and the last years of the 19th form an epoch that will be marked in the annals of medicine by its immense progress in elucidating the etiology and the mode of transmission of tropical diseases and in deducing therefrom measures to prevent them. Inductive and deductive epidemiology, in searching for sources of infection, recognized rather early in this period the relationship of three genera of mosquitos to the transmission of filariasis, malaria and yellow fever, the rôle of ticks in Texas fever and the tsetse fly in African sleeping sickness. In this era also falls the first experimental proof of insect transmission of a plant disease: in 1891, Waite showed that bees and wasps, while visiting pear blossoms in search of nectar, are active vectors of bacteria causing fire blight of pears.

But at the beginning of this period the concept of infection chains; in fact, the biologic approach to the solution of the living contagia, had not been formulated. Medically oriented microbiology had relatively little appreciation then of the vast implications of a host-parasite relationship. It dimly visualized, however, that the serial transmission of certain infective agents from host to host in all probability is of boundless ancestry. Indeed, the concept of insect transmission of human disease has ancient roots, but since experimental proof was invariably lacking, the concept failed to take its proper place in scientific thought. With the exception of the study of insects in relation to man and his direct economic welfare, little was known of entomology until it became associated with the pathogenic action of insects on man, and then medical research stimulated entomology, and entomology stimulated medical research. A graduate of the University of California, G. H. F. Nuttall, in a classical and historically significant paper "On the Rôle of Insects, Arachnids and Myriopods as Carriers in the Spread of Bacterial and Parasitic Diseases of Man and Animals", written in 1899, described the situation at that time in these words: "Very few works on hygiene even mention the rôle of insects as carriers of infection, and those that do generally speak vaguely on the subject." But since that statement was made, many new facts have been brought to light to dispel the vagueness; arthropods have proved to be of prime importance in medical, zoological and plant science; and control of insects has assumed its rightful sound position among hygienic measures against a great many infectious diseases.

In an interesting logical sequence, the experimental evidence that insects are concerned, and quite vitally, in the transmission of disease was brought to light with the filarial worm causing elephantiasis in man; with the protozoan parasites causing human malaria and the cattle diseases—Texas fever and nagana; and

[1] Read on October 10, 1952, at the opening ceremonies of the Biochemistry and Virus Laboratory Building of the University of California at Berkeley.

finally with the viral agent of yellow fever. The rôle of poikilothermic hosts in the ecology of human infections, of animal plagues and in the spread and development of plant diseases—components of the so-called heterogenous infection chains—was then made brilliantly clear. With increasing frequency insects or arthropods have stood out as the connecting bond between two other links of the chain. The two end links, the infected patient, animal or plant reservoir host and the uninfected person, animal or plant, may be connected by an intermediate, the arthropod or other invertebrate.

For the sake of convenience, the word "vector" (from the Latin word *vehere*, to carry) is now commonly used to label the middle link—the invertebrates indispensable to the successful passage of certain disease agents from the infected, not necessarily the sick, to the well. Use of the term is usually restricted to those arthropods which occupy this position. However, it must be emphasized that they are not always merely carriers, they may be definitive hosts or reservoirs as well.

Infections of this type are not transferred through insects in general; they depend on arthropods—mosquitos, ticks or mites—belonging to specific genera or species. Thus between the infective agent and the poikilothermic host a highly specific interrelationship, a fundamental character of parasitism, is fully established. The primary purpose of much work on the problems of vectors, because of its obvious public health and economic importance, has been to identify the insects responsible for transportation of infectious agents and to recognize, and if possible to control, the factors that may influence their ability to transmit. The terms "vector-" and "arthropod-" and "insect-borne disease" are used today to avoid lengthy circumlocutions.

Although human and animal pathology have much in common with plant pathology, the basic thought in these fields is rarely compared analytically, even though casual scrutiny of the published records shows that there are remarkable general similarities in the phenomena associated with insect transmission of animal and plant diseases. And, as one would expect, there are also significant differences. Virus diseases constitute the largest group of arthropod-borne plant diseases, while diseases caused by protozoa lead the list of vector-borne animal diseases, followed closely by the rickettsias and the viruses. The specificity of the relationships between vector and disease agent in both groups varies widely. Many of the highly specialized relationships characterize virus diseases transmitted by insects sucking blood or plant sap. These similarities and differences and other factors to be reviewed make it imperative that vector and reservoir problems in virus disease must be discussed in the light of the contributions made by both animal and plant pathology.

Through years of tedious field and laboratory observations it has been recognized that certain definite requirements must be met before it is scientifically correct to conclude that an arthropod harbors and distributes a viral agent. Fulfilling the following requirements is accepted as adequate proof of arthropod transmission of a viral infection to either animals or plants.

(1) A close, not necessarily constant, association of an arthropod with the clinically or subclinically infected.

(2) Regular visits by the insect to the healthy under conditions suitable for exchange of the virus.

(3) The presence of the infective viral agents in the arthropod in nature or in the infected, but again not necessarily the sick.

(4) Experimental production of the infection, or preferably the disease, by the arthropod, through feeding or visits under controlled conditions, with adequate checks.

But to form a complete picture of the disease as it is being carried on under natural conditions, to these general requirements must be added the elucidation of the rôle of arthropods as true reservoirs as well as vectors of the viral agent. Man and many other species of vertebrates serve as only aberrant hosts for the viruses of yellow fever, louping ill and Colorado tick fever. The vertebrate host may be regarded in these infections, just as in Nairobi sheep and goat disease, African catarrhal fever and horse sickness, as only a temporary repository and as disseminator of virus to the true reservoirs—the "vector" mosquitos and ticks. There is considerable evidence that a similar multiple host relationship maintains dengue, phlebotomus fever, many of the arthropod-borne encephalitides and Rift Valley fever. But the certain identification of the true reservoirs of these diverse viruses has not been settled. The recognition that the clover club leaf virus can maintain itself indefinitely in the insect vector (Black, 1950) establishes the existence of true arthropod hosts of a plant disease. The biologic implications of the recent observation that elementary particulate plant viruses can multiply in insect tissues leads into a whole new area of thought. The mere fact that a virus is capable of multiplication both in plant and in arthropod hosts and that its transmission depends directly on a specific insect deserves decidedly more than casual notice by the student of animal viruses. Knowledge concerning the plasticity of viruses in adapting to diverse host tissues is indeed prerequisite to an understanding of their possible position as symbionts or mutualists in the arthropod host.

Surprisingly few animal diseases are induced by viruses transmitted by arthropod vectors, and these vectors are limited to certain species of mosquitos, ticks and mites. In sharp contrast, there are in the neighborhood of 150 known virus diseases of plants; insects or vectors belonging to five orders of arthropods are responsible for the transmission of most of these.

The natural arthropod vectors of animal viruses are found in the following two orders: *Diptera*, with families *Psychodidae* and *Culicidae*, and *Hemiptera*, with the class *Arachnida*, superfamilies *Ixodoidea* and *Parasitoidea*. Except for the filth flies (*Muscidae*, *Calliphoridae* and others) and possibly the louse, thought to be passive carriers of the poliomyelitis virus, those endowed with piercing sucking mouthparts and feeding on blood are the principal vectors. Although not infrequently only one or two species are infected in nature, many other species of both orders may be found capable of experimental transmission of the human or mammalian virus by bite. Just as the spectrum of susceptibility to animal viruses of diverse mammalian and avian artificial hosts has invariably been proven broader than that of the spontaneous hosts, so the species specific acceptance of a viral agent by arthropods is probably less limited than the published trials indi-

cate. Whenever, for example, a search for possible accessory vectors of certain encephalitis viruses has been undertaken, ticks and even triatomas have been proven to act as transmitting agents. The relationship between the virus and the artificial insect hosts may be so intimate that the virus is transmitted transovarially to the next generation.

The insect vectors of virus diseases of plants are found in five orders: (1) The *Orthoptera*, or grasshoppers, which have chewing mouthparts, are, as a rule, not very effective vectors. (2) *Thysanoptera*, which includes the thrips, feed with a rasping, sucking action and are involved in specific biological transmissions. (3) Approximately 90 per cent of all insects concerned in the transmission of plant viruses are members of the *Homoptera*. They all have piercing sucking mouthparts and feed exclusively on plant sap. They are especially effective vectors. This order includes the more than twenty different species of aphids concerned in the transmission of a considerable number of different viruses. Next to the aphids in rank as virus vectors are the leaf hoppers. The plant hoppers or lantern flies include several important vectors, while the white flies, scale insects and mealy bugs transmit few viral diseases. (4) The *Hemiptera*, or plant bugs, have piercing sucking mouthparts, but few have been incriminated in the transmission of plant viruses. (5) The *Coleoptera*—the beetles and weevils—all chewing insects, play quite a subordinate rôle in virus transmission.

This being true, it must be concluded that the sucking insects as a group are much more effective vectors than the chewing insects. There is, of course, variation within the group, for not all sucking arthropods are equally effective. Some seem unable to transmit the virus at all or they transmit it only mechanically and incidentally. Others transmit it so effectively and with such regularity that the relationship clearly represents a highly specialized adaptation of the virus to the arthropod host.

The newer ideas about the modes of virus transmission are patterned after the basic studies on yellow fever and the classification proposed by Huff (1931). With few exceptions the sucking arthropods must bite or puncture in order to transfer the virus. Insect transmission of plant viruses without wounding plant tissues is unknown. However, it is suspected that, in certain tick-borne human and animal virus infections, through the introduction of infectious dejecta or crushed insects into scratch wounds or even the inhalation of dust from the fleece of sheep contaminated with such materials, infection chains may be established.

At present the most intriguing entomological work is concentrated on the factors that influence the vector's ability to transmit. The adjectives "external," "internal" and "biological" are being used, with many different connotations, to describe transmission. It is external when the virus is carried as a contaminant on the exterior of the arthropod. Avian pox has been so transmitted by *Stomoxys calcitrans*, *Liponyssus* mites and several species of mosquitos, while myxomatosis of rabbits is apparently dispersed over wide areas in Australia by blood-sucking *Diptera* with externally soiled mouthparts. Certain vectors of a select group of plant viruses can infect healthy plants immediately after leaving a diseased one.

The original view that the usual transmission is external had to undergo definite changes when it was realized that fasting before and after a short infective meal greatly increased the percentage of transmissions. The term "internal mechanical transmission" was then introduced to accommodate passage through, but not multiplication of the viral agent within, the arthropod host. The viruses are sucked up through the vector's mouthparts, not just carried on the outside, but how they get into the position from which they can be injected into another plant is unknown. The short time elapsing between feeding on an infected plant and infecting a healthy one seems to preclude the penetration of the gut wall, circulation in the blood and entry into the salivary glands, as is the case for the so-called persistent plant viruses. Internal mechanical transmission is well recognized in plant pathology, but has been proven only in poliomyelitis and C virus in animal pathology. Even though blowflies—*Phorma regina* and *Phaenicia sericata*—are not true hosts of either poliomyelitis or C virus, the persistent excretion of these agents for many days after exposure suggests the possibility of contamination of food by fly excreta (Melnick and Penner, 1952).

Biological transmission is considered proven when it is known that one or more of the following relationships exists between the arthropod and the virus: (1) An extrinsic incubation period between the time the insect feeds on infected plants or animals and the time it becomes infective. (2) An obligatory relationship between the insect and the virus it transmits. (3) Multiplication or increase of the viral agent in the arthropod's body. (4) A relationship between the life stage of the arthropod and its ability to transmit the viral agent. (5) Hereditary transmission of the virus through the eggs to succeeding generations.

(1) *The extrinsic incubation period.* Already in the days of the Yellow Fever Commission it had been recognized that the mosquito *Aedes aegypti* could transmit this disease by bite only after a period of 9 to 12 days had passed after an infective feeding. The virus ingested through the first bite was found throughout the body of the susceptible mosquito studied, but it did not transmit the agent by bite until this length of time had passed. These mosquitoes were held at temperatures between 74° and 90°F.

Several explanations have been offered of what course the virus takes through the arthropod during this time. There is general agreement that this extrinsic incubation period is the time required for the virus to complete its cycle through the mosquito—through the stomach into the intestine, through its walls into the coelom fluid and on into the salivary glands, whence it can be introduced into another animal or plant. The virus content of the mosquito drops sharply during the first days after its blood meal, but then it begins to rise, sometimes to quantities exceeding that originally ingested.

This cycle is subject to several influences—the environment of the arthropod, the nature of the virus and the nature of the arthropod. Temperature, humidity, sunlight and the amount of flight profoundly influence the physiology of the mosquito. The higher the temperature within limits, the shorter the period. The cycle will be completed only if the mosquito is healthy and is held under optimal environmental conditions. The careful quantitative studies of the yellow fever

virus convincingly correlate this incubation period with multiplication of the virus.

(2) *The obligatory relationship.* There are numerous well-demonstrated cases of the specificity of the relationship between arthropod vectors and the viruses they transmit. The most striking examples are the plant virus infections biologically transmitted by leaf hoppers. Some plant viruses are so specific in their vector requirements that even different strains of one virus may have different vectors. Whether the now apparent extreme specificity of some is real, however, is impossible to say, for with many viruses only a few species have been tested as vectors. For example, the preëminence of *Myzus persicae* as a common vector may be less real than it appears. It is commonly the first insect to be tried as a vector of a newly discovered virus and if it succeeds, others are not tested. Its wide host range, however, makes it peculiarly important as a field vector of many different viruses. The evidence in support of high specificity is generally negative, and there is always the possibility that additional vectors will be discovered. When animal viruses are transmitted by representatives of the family *Ixodidae*, specificity, as far as has been investigated, is sharply evident. For example, the virus of Russian encephalitis is transmitted by *Ixodes persulcatus*, thought to be the only tick involved in the Far Eastern form of the disease; the louping ill agent is transmitted by *Ixodes ricinus*, and Colorado tick fever by *Dermacentor andersoni* and *Dermacentor variabilis*. The virus is transmitted to man or animals through the saliva of the mosquito as it bites. It is well to remember that the relationship between the arthropod and these agents is so intimate that transovarian passage assures continuous passage of the virus from host to host. In laboratory experiments of unknown practical application, the Russian virus has been found to survive in *Dermacentor*, *Rhipicephalus*, *Hyalomma*, *Haemophysalis* and even *Ornithodorus* ticks (Chumakov *et al.*, 1945). In a few experiments *Aedes vexans* var. *nipponii* and *A. excrucians* accepted the virus of tick-borne encephalitis, but it persisted for only 5 days, showed no increase and was not transmitted by mosquitos. Thus it must be concluded that the agent is possibly a parasite of ticks (Levkovich and Gutzevich, 1941).

In contrast, the yellow fever agent is much less discriminating—it may be readily transmitted by South American and African mosquito species of the genera *Aedes*, *Haemogogus* and possibly by one of the vast tribe of *Sabethini*. It is quite interesting that, aside from mosquitos, ticks (*Acarinae*) are better able to retain the virus of yellow fever than are any other group of arthropods studied. This, coupled with the fact that ticks can harbor other viruses and can transmit them transovarially, should focus experimental attention on this arthropod. Although the mosquito is considered the reservoir as well as the vector of this virus, because once it is infected it remains so and can transmit the agent again and again through its life span, this reservoir is effective for only a relatively short time. Repeated transfer throughout the year—essential to prevent the dying out of the virus—necessitates an adequate supply of vertebrates susceptible to the infective agent. Whether such a supply is available in the areas where the immunity level among primates is known to be high has not yet been

determined. In this connection, the over-all relationship of viruses to ticks may be significant.

The studies on the vector-borne encephalitides deserve brief consideration. Just as in yellow fever, the host range is specific, but in the encephalitides it is also broad. The importance of three genera of mosquitos—*Culex*, *Aedes* and *Culiseta*—in the transmission of equine encephalomyelitis, St. Louis and Japanese encephalitis has been amply confirmed. Both the Western equine and the St. Louis virus have been found in naturally infected *Culex tarsalis*, *Culex pipiens*, *Aedes dorsalis*, *Culex quinquefasciatus* and *Culex stigmatosoma* (Reeves, 1951); the Japanese B agent in *Culex tritaeniorhynchus* and the Eastern equine virus in *Mansonia perturbans* and *Culiseta melanura*. Likewise the virus has been recovered from mites and even chicken lice (*Eomenacanthusa stramirseus* and *Menopon pallidum*) in chicken coops (Howitt, 1948; Howitt *et al.*, 1949). In the laboratory several species of *Culex*, *Aedes*, *Theobaldia* and *Psorophora* not only became infected, they transmitted the viruses. Under experimental conditions not only the ticks *Dermacentor andersoni* and *Dermacentor variabilis*, but also the cone-nosed bug, *Triatoma sanguisuga*, can act as transmitting agent and as a reservoir host of the Western equine virus. The susceptibility of these experimental hosts attests merely to the great adaptability of the virus or viruses; it fails to elucidate how the virus is ordinarily propagated and maintained. Again as in yellow fever, it has never been possible to transfer the virus from one mosquito to another experimentally. Coupled with the recognized broad host spectrum, the cyclic appearance and disappearance of the infection in certain areas, particularly during the winter months, indicate that the arthropods, though efficient and specific vectors, simply cannot maintain the infective agent without susceptible vertebrate hosts. Certain broadly surveyed domestic and several species of wild birds are now accepted as important sources of vector infection. But since no evidence of chronic infection or carrier-reservoir stages has been secured, the ecological situations that maintain the virus remain obscure. Preliminary observations suggest that the long survival of the virus may be ultimately found to be supported by birds and that dispersion may in some way be connected with the newly hatched. Whatever the ultimate solution may be, it is already evident that, whenever several genera or species of arthropods are capable of transmitting a virus, the ecology of the latent or apparent infections they are capable of producing in vertebrates appears to be exceedingly complicated.

The nature and cause of the specificity of the relationship between certain arthropod vectors and the viruses they transmit have never been satisfactorily explained. Within single species of insect, single strains or races may be unable to transmit a certain virus. The essential differences between the races apparently lie in the unequal permeability of the intestinal walls of the insect vectors (Storey, 1933). However, it is not unlikely that individual differences in the ability of vectors to transmit viral agents may be genetic, a simple inherited Mendelian recessive factor. This has already been proven for two species of mosquitos in relation to the bird malarial parasite (Huff, 1931).

The vector transmission is considered obligatory when a virus has no other

means of transport, of species survival. Animal pathology furnishes no illustrative examples of this circumstance, but plant pathologists have listed over 30 viruses transmitted specifically and exclusively by sucking insects and not transmissible mechanically by plant extracts. The idea that insect transmission of some viruses is obligatory is obviously based on negative evidence, and there is always the possibility that a suitable technique for artificial transmission will be discovered.

(3) *Multiplication of the virus in the arthropod.* One of the foremost current problems under study by workers in the virus field deals with the question of the activities of the virus in the arthropod. The descriptions of the existence of the yellow fever virus in mosquitos differ largely because it is not realized that the effect of temperature is decisive in determining not only the length of the extrinsic incubation period, but also the concentration of the virus in the vector. They differ too because the selection of different species of mosquito and virus strains may doubtless envision conditions that do not represent the true picture of the relative efficiency of certain vectors in their natural environment. Furthermore, as might be expected, a yellow fever virus maintained artificially in unusual tissues loses its capacity to establish itself and grow in mosquitos. Finally, it is fully established that mosquitos fail to become infected if the blood meal contains less than 1,000 M.L.D. of virus for mice. To insure a 5 to 25 per cent infection rate of fed mosquitos, at least 20,000 to 30,000 M.L.D. in the blood of an animal early in the infection, when viremia is intense, must be ingested if the virus is to survive and multiply. In all probability, the ability to transmit the virus by bite is related to the general level of the virus in the mosquito, not to infection of the cells of its salivary glands. Measurement of the exact amount of virus transferred is quite difficult, but the available data indicate that at least 100 infectious doses for monkeys, if not more, must be injected by the vector into the susceptible host in order to insure infection (Whitman, 1951).

The biologic relationship between the dengue virus and its insect vectors has been little studied. Until recently the only suitable experimental host was the monkey. Notwithstanding this limitation, there is some evidence that the virus does develop in the mosquitos. An extrinsic incubation period of 11 to 14 days is required after an infective blood meal before the arthropod can transmit the agent and can function as an effective vector for the rest of its life, 1 to 3 months or more.

That a virus derived from large mammals other than man and capable of producing viremia is transmitted by an arthropod was first demonstrated with the Western equine encephalomyelitis agent (Kelser, 1933–1936). A number of workers have adduced the following evidence that Eastern and Western encephalitis viruses proliferate in the tissues of *Aedes sollicitans* and *A. aegypti*: (1) The virus is well distributed throughout the tissues of the infected mosquitos. (2) The agent may multiply a thousandfold or ten thousandfold within the mosquito. (3) At suitable temperatures the mosquito becomes infective about 7 to 20 days after the infecting meal and remains so for the rest of its life (Merrill *et al.*, 1935). Two equally significant observations relating to the development of the encephalitis viruses in the vector are rarely mentioned: (a) The Western

equine agent increases in titer when cultured in the presence of mosquito tissue (Trager, 1938). (b) The vector seems not to be adversely affected by infection with the Eastern virus. Just as with the yellow fever virus, there is no transmission from mosquito to mosquito.

The relationship between a virus and the tick is more intimate. The experimental observations are confined to the Russian Far East or verno-aestival encephalitis, louping ill and Colorado tick fever. These are transmitted transovarially and can cause infections in the larvae, nymphs and subsequent adult life cycle stages, although virus may not be detected in the eggs of the originally infected adults (Florio *et al.*, 1950). Detailed studies with the Russian spring-summer encephalitis agent have shown that it is extensively distributed and has penetrated deep into all organs of the tick vector. The virus appears to remain undiminished during at least 25 days in the gut of the tick. In the genital organs the viral content at first decreases, but later increases again. Concentrations are high in the salivary gland and some virus is also found in the brain. The integument of the tick is free of the agent. The virus passes into the egg by penetrating the walls of the ovary and thence entering the developing ovum. Finally the virus is preserved through the winter in the hibernating ticks. Although the increase in virus that follows an infective meal has not been measured, the intimate biologic adaptation between the agent and the vector *Ixodes persulcatus* amply attests to the conclusion that the virus does multiply in arthropod tissues (Pavlovsky and Soloviev, 1940). When this virus is less adapted to a vector, as, for example, to *Haemophysalis concinna*, it multiplies little, if at all; this tick will induce merely subclinical infection in the highly susceptible mouse (Kozlova and Soloviev, 1941).

Until recently, information about the proliferation or multiplication of plant viruses in their insect vectors was decidedly contradictory. In fact, the evidence was largely circumstantial, since few quantitative studies had been carried out, and the observations were subjected to different interpretations (Bawden, 1950). Since no significant differences in morphology, disease symptoms or longevity and power of procreation between infective and noninfective individuals of arthropod species have been observed, one plant pathologist accepted this absence of reaction as proof that multiplication would not occur in the body of the incriminated vectors. The general biologist, however, recognized long ago that with few exceptions (typhus and plague), arthropods suffer very little under the influence of the parasites when they serve as vectors. Even the massive changes invoked by the formation of oocysts on the ventriculum of *Anopheles* mosquitos or by the invasion of the ovaries of ticks by spirochaetes have no influence on the nutrition, locomotion and reproduction of the arthropods. The paramount significance of these observations is that the parasite does not interrupt the continuity of the insect species. Should the parasite in some way endanger the life span of the arthropod or disturb its desire to feed, then its chances to transmit the infective agent would be diminished. It would be a negation of biologic principles to find a parasite persisting long in so unfavorable an environment. The arthropod would then serve neither as an efficient vector, nor as a reliable

reservoir. Such situations have not been observed in animal pathology, and it is most unlikely that an exception is made in the transmission of the parasites that cause plant diseases.

Although it is well known that the viruses of certain plant diseases persist in the body of the arthropod for a relatively long time and that some are even transmitted through the eggs, only limited crucial experiments in producing a passage series in order to develop infective insects by direct mechanical inoculation of virus, as carried out, for example, with the Eastern equine virus, have been undertaken. A few months ago, Maramorosch (1952) extended previous observations that the leaf hopper, *Macrosteles divisus*, the vector of aster yellows, may be readily infected mechanically by injection of extracts from infected insects. The concentration of the virus did not diminish by direct passage from insect to insect. In fact, it multiplied to such an extent that the arthropod of the 8th passage proved infective after a minimum incubation period characteristic for a virus concentration of 10^{-3}. If the virus had not multiplied, the dilution of the virus after the 8th insect passage would have been 10^{-40}. In this connection it is well to remember that the highest dilution end point for the animal encephalitis viruses in tissues is 10^{-10} and that the tobacco mosaic virus in plant juice has a dilution end point of 10^{-7}.

Three additional plant viruses—the leaf-hopper-transmitted wound tumor (Black and Brakke, 1952), corn stunt (Maramorosch, 1951) and clover club leaf virus (Black, 1950)—none of them mechanically transmissible from plant to plant, have been proven to be transmissible to insect vectors by injection. The biological importance of these findings is doubtless far-reaching, since the infective agents cannot be considered as plant viruses only. Their perfect adaptation to the insect vectors characterizes them as connecting links between plant and animal viruses. If one can draw an analogy with the animal viruses, their origin may be in the insects, not in the plants. Further evidence that certain plant viruses multiply in their leaf hopper vectors has rapidly accumulated from experiments on passage of the virus through the egg for many generations (Fukushi, 1940; Black, 1950) and from titrations showing virus increase in insects during the incubation period (Black, 1941 and 1950). These experiments also recall the circumstantial evidence of multiplication of the virus of aster yellows in its vector by an ingenious method of thermal inactivation of the agent. By exposing the body of infective leaf hoppers to temperatures of 31° to 32°C. for a sufficiently long time, the virus is destroyed; but if the exposure is short, the insect only temporarily loses its ability to transmit the virus. The spontaneous regaining of the virus is interpreted as a multiplication of the small quantity of the virus not destroyed by the shorter heat treatment. Thus far, virus multiplication has been demonstrated only in the body, particularly in the blood of leaf hoppers, and one awaits with keen interest the results of experiments with certain aphids which transmit certain viruses biologically, but are equally capable of transmitting others in a purely mechanical manner (Maramorosch, 1949).

(4) *Relationship of the stage of the insect to transmission.* Certain vectors are unable in the nymph or larval stage to transmit plant viruses. The explanation

must be sought either in the anatomy or physiology of the digestive system of the arthropod or in the incubation period. The nymphs of *Macrosteles divisus* are often unable to transmit the virus of aster yellows because the period required for the insect to become infective is longer than the period of metamorphosis (Kunkel, 1926).

(5) *Transovarian passage.* Despite the intricate biological relationship between viruses and their arthropod vectors, the congenital generation-to-generation transmission of viral agents appears to be the exception, not the rule. Hard ticks, which have a three-host life cycle, transmit viruses transovarially and from stage to stage. The biologic relationships between the viruses of Russian encephalomyelitis or Colorado tick fever and their vectors have not been studied in any detail. Of interest is the fact that in *Dermacentor variabilis* ticks experimentally infected, a generation-to-generation transmission of St. Louis virus was accomplished to the third generation. All the stages of the tick including the eggs remained infected after being stored for 10 months (Blattner and Heys, 1941). Furthermore, infected ticks may be stimulated to complete their life cycle in a shorter period than normal ticks (Syverton and Berry, 1941). Penetration of the walls of the ovary and thence entrance of the virus of Russian encephalitis into the developing ovum has been demonstrated in *Ixodes persulcatus* (Pavlowsky and Soloviev, 1940). Stage-to-stage and generation-to-generation transmission of the virus of lymphocytic choriomeningitis by *Dermacentor andersoni* has been reported, although attempts to transmit it by feeding the infected adult ticks were unsuccessful (Shaughnessy and Milzer, 1939). Numerous studies by very competent investigators have failed to confirm early claims that a specimen of *Aedes aegypti* bred from the eggs of an infected female transmitted yellow fever (Whitman, 1951). In fact, as already pointed out, generation-to-generation transmission of viruses has never been proven in *Culicidae* mosquitos.

The only known instance of transovarian transmission of a plant virus is in the stunt disease of rice. A number of Japanese workers (Takami, 1936) contributed to the first conclusive revelation that a plant virus disease is transmitted by a leaf hopper (*Nephotettix apicalis*). Later, carefully conducted experiments indicated that the Oryza virus is transmitted through the eggs to the nymphs. When infective females were paired with infective males, the majority of the offspring in all progeny was infective. When infective females were paired with noninfective males, both infective and noninfective progeny were found. But when noninfective females were paired with infective males, none of the progeny was infective (Fukushi, 1939). Since leaf hopper vectors transmit viruses biologically, there is reason to suspect that other species may transmit the agent from generation to generation. Another fruitful field of comparative research suggests itself.

This sketch of some essential facts concerning vectors and reservoirs of virus diseases cannot be closed without a brief consideration of certain epidemiologic implications. The question is frequently raised as to the propriety of applying the term vector to arthropods and the term host to vertebrates and plants.

Viewed from the aspect of the infection or the disease in man and animals and plants, the arthropod does play the vectorial and carrier rôle. But viewed from the aspect of the virus and its requirements, the mosquitos and ticks qualify as definitive hosts or reservoirs better than do the vertebrates. The virus finds more permanent habitation in the so-called vector than in the vertebrate, where its life is fraught with many hazards. It must be rescued from the vertebrate by the arthropod within an infinitesimal period in order to survive. The plant virus-vector relationship is not as demanding on the arthropod because the infected plant serves well as a chronic virus carrier. With few exceptions the arthropod is not required to serve as a reservoir. However, common to both the animal and the plant vector-borne viruses are their extremely plebeian nutritive requirements. The continuous change from hosts as diverse as mammals and birds or plants to the tissues of arthropods must entail either a well-organized adaptation of all metabolic activities or relatively simple nutritive requirements. It is anticipated that the application of modern tissue techniques not only may furnish information about these problems, but may explain the basis for the complete tolerance of the arthropod to the highly virulent animal or plant viruses they transmit.

A consideration of the epidemiology of vector-borne virus infections must finally call attention to the discovery of new animal viruses through the systematic inoculation of crushed arthropods into mice. Members of the International Health Division of the Rockefeller Foundation have isolated two new viruses from human beings and fifteen from mosquitos. There is strong suggestive evidence that these viruses arose from the fauna of tropical forests which appear to furnish environmental conditions singularly endowed to favor the development and perpetuation of agents potentially pathogenic to human beings. Nine of the viruses originated in Africa and eight in South America. They are at present the subject of comprehensive study which may ultimately lead to a better understanding of their characteristics and properties when compared with a selected group of well-known viruses. Systematic testing of arthropods for viral agents by mouse inoculation has led to the isolation of another new virus which may be of importance as the cause of encephalitis in this State.

The study of arthropod vectors has told new secrets, but others hold themselves in reserve. It is well to remember

"Naturam expellas furca, tamen usque recurret
Et mala perrumpet furtim fastidia victrix."

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THE EFFECT OF PYRIMETHAMINE (DARAPRIM) AGAINST *PLASMODIUM GALLINACEUM* INFECTIONS IN CHICKS

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Pyrimethamine, 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, is one of a large series of diaminopyrimidines and related compounds shown to have antimalarial activity against blood-induced *Plasmodium gallinaceum* in chicks and *P. berghei* in mice. The activity of pyrimethamine against the former infection has been confirmed by Schneider *et al.*, 1952. The compound was found to be effective in extremely small doses against blood-induced *P. cynomolgi* (Schmidt and Genther, 1953), and *P. knowlesi* (Jaswant Singh *et al.*, 1951) in monkeys.

Falco and co-workers (1951) found that pyrimethamine had some prophylactic activity against sporozoite-induced *P. gallinaceum* infections in chicks. Jaswant Singh *et al.* (1952) showed the compound to be completely prophylactic against this infection at a dose of 0.3 mg./kg. twice daily for four days. Pyrimethamine has marked prophylactic activity against sporozoite-induced *P. cynomolgi* infections in monkeys (Schmidt and Genther, 1953). Goodwin has summarized the information available on the activity of the antimalarial against naturally acquired human malaria. In definitive tests employing prisoner volunteers, Coatney *et al.* (1952 and 1953a) found that one dose of pyrimethamine produced long periods free of relapses from sporozoite-induced Chesson strain *P. vivax*, and that small weekly doses (25 mg. per man per week) for eight weeks resulted in suppressive cures.

A comprehensive study of this compound against *P. gallinaceum* was undertaken by us in October 1950, shortly after we learned of its pronounced effectiveness. The drug was tested in single and multiple doses for suppressive activity against blood-induced infections, and for prophylactic and curative activity against sporozoite-induced infections. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Week-old New Hampshire red chicks weighing 45 to 55 grams were used in all the experiments. For the blood-inoculated series the BI strain of *P. gallinaceum* was used and for the sporozoite-induced infections the SP strain of the parasite was used (Greenberg *et al.*, 1950). Inoculations of parasites were done according to the methods described in detail elsewhere (Coatney *et al.*, 1953). All treatments were administered orally. At concentrations which were soluble in aqueous or dilute lactic acid solutions, the compound was administered by syringe and catheter. At dosages which were not soluble in conveniently small amounts of solvent, the drug was administered in gelatin capsules.

The treatment schedules employed and the appraisal of the effects of the treatment will be described in the section on results.

RESULTS

When tested by the usual procedure used in this laboratory for determining suppressive activity of antimalarial compounds (Coatney *et al.*, 1953) pyrimethamine was active at doses equal to or more than 0.00001 to 0.00002 mg./gm. twice daily for 4 days beginning 4 to 5 hours before inoculation of parasites.

When the compound was administered in a single dose 4 to 5 hours before inoculation (Table 1) it had a discernible effect, as determined by comparing the third day parasitemia in the controls and in the treated chicks, at dosages equal to or more than 0.00025 mg./gm. The chicks in this experiment were examined for parasitemia from the second to the tenth day after inoculation. At no dosage below 0.001 mg./gm. were parasites reduced below microscopically detectable levels at any time during the observation period. In chicks receiving 0.001 mg./gm. parasites were cleared by the third day, but reappeared, on an

TABLE 1

The effect of single doses of pyrimethamine against blood-induced P. gallinaceum *infections in the chick. Treatment: 4 to 5 hours before inoculation of parasites*

DOSAGE OF PYRIMETHAMINE (MG./GM.)	MEAN 3RD DAY PARASITEMIA (PARASITIZED RBC/10^4 RBC)	MEAN DAYS TO CLEAR PARASITES	MEAN DAYS TO REAPPEARANCE OF PARASITES
None	6350		
0.000016	5260		
0.000031	4800		
0.000063	5240		
0.000125	5000		
0.00025	1478		
0.0005	13		
0.001	6	3.0	3.8

average, 3.8 days later. This dosage, then, was the minimum amount which would "clear" parasites even temporarily.

The effect of a single dose of pyrimethamine of 0.002 mg./gm. administered the day of, or 1 to 7 days before inoculation of parasites was next examined (Table 2). A significant effect, as determined by third-day parasitemia, was observed when the drug was administered four or fewer days prior to inoculation. Parasites were "cleared" only when the drug was administered the day of or the day before inoculation of parasites. The effective period of the drug (days to "clear" parasites plus days before parasites reappeared) was about 8.4 days when the drug was administered the day of the inoculation and 6.0 days when administered the day before.

When tested by the usual procedure used in this laboratory for determining prophylactic activity of compounds in sporozoite-induced infections, pyrimethamine was partially prophylactic (produced a delay in appearance of parasites of at least 2 days) at a dosage of 0.00003 mg./gm. twice daily for 4 days (Table 3). It was completely prophylactic, aborting the infection in most chicks, at a dosage of 0.00025 mg./gm. Of the 15 treated chicks which became infected in

this latter experiment, only 5 died of exoerythrocytic infections, compared to 9 of the 10 untreated chicks which died with these forms of the parasite present in smears of the brain.

Single doses of pyrimethamine, administered 4 to 5 hours prior to the inoculation of one mosquito equivalent of sporozoites (Table 4), were partially prophylactic at a dosage of 0.005 mg./gm. and completely prophylactic at 0.004

TABLE 2

The effect of single doses of pyrimethamine (0.002 mg./gm.) against blood-induced P. gallinaceum *infections in the chick*

DAY OF TREATMENT*	MEAN 3RD DAY PARASITEMIA (PARASITIZED RBC/10^4 RBC)	MEAN DAYS TO CLEAR PARASITES	MEAN DAYS TO REAPPEARANCE OF PARASITES
Untreated	3470		
−7	4060		
−6	3920		
−5	3580		
−4	1616		
−3	264		
−2	22		
−1	4	2.6	3.4
0	6	3.4	5.0

* Day 0 = day of inoculation.

TABLE 3

The effect of pyrimethamine against sporozoite-induced P. gallinaceum *infections in the chick. Treatment: twice daily for 4 days beginning 4 to 5 hours before inoculation of sporozoites*

DOSAGE OF PYRIMETHAMINE (MG./GM.)	NO. CHICKS INFECTED/ NO. CHICKS INOCULATED	PREPATENT PERIOD (DAYS)*	NO. CHICKS DYING WITH EE FORMS
None	10/10	8	9
0.000031	5/5	10	2
0.000063	5/5	12	3
0.000125	4/5	16	0
0.00025	1/5	∞	0
0.0005	0/5	∞	0

* Day on which 50 per cent or more of the chicks exhibited parasitemia.

mg./gm. Ten of the 14 infected chicks which were treated died with exoerythrocytic parasites present in the brain.

Single doses of the drug administered 7 days after the inoculation of sporozoites prevented death from exoerythrocytic parasites even at a dosage of 0.001 mg./gm. (Table 5). In this series only one treated chick died, 35 days after inoculation; exoerythrocytic parasites were detected in its brain smear. This chick had received the highest dose of pyrimethamine administered. It should be noted also that all doses tested "cleared" parasites temporarily. Both the time required for clearing parasites as well as the number of days before parasites reappeared

were a function of the dosage. The higher the dosage of the drug the shorter the time to clear parasites and the longer the time to the reappearance of the parasite. However, the total period over which the drug exerted its effect was 4.6 days at 0.001 mg./gm. as compared to 7.2 days at 0.016 mg./gm. A sixteen-fold increase in the dosage increased the drugs effective period only 55 per cent. Furthermore, even at the highest dosage levels administered, no cures were achieved.

TABLE 4

The effect of single doses of pyrimethamine against sporozoite-induced P. gallinaceum *infections in the chick. Treatment: 4 to 5 hours before inoculation of sporozoites*

DOSAGE OF PYRIMETHAMINE (MG./GM.)	NO. CHICKS INFECTED/ NO. CHICKS INOCULATED	NO. CHICKS DYING WITH EE FORMS	PREPATENT* PERIOD
None	10/10	10	7
0.0005	5/5	5	10
0.001	5/5	2	12
0.002	3/5	3	14
0.004	1/5	0	∞
0.008	0/5	0	∞
0.016	0/5	0	∞

* Day on which 50 per cent or more of the chicks exhibited parasitemia.

TABLE 5

The effect of single doses of pyrimethamine against sporozoite-induced P. gallinaceum *infections in the chick. Treatment: 7 days after inoculation of sporozoites*

DOSAGE OF PYRIMETHAMINE (MG./GM.)	NO. CHICKS CLEARED/ NO. CHICKS INOCULATED	MEAN DAYS TO CLEAR PARASITES*	MEAN DAYS TO REAPPEARANCE OF PARASITES*	NO. CHICKS DYING WITH EE INFECTIONS
None	0/10	—	—	10
0.0005	1/5	3	1	0
0.001	3/5	3.6	1	0
0.002	4/5	3.5	2	0
0.004	5/5	2.8	3.4	0
0.008	5/5	2.6	4.6	0
0.016	5/5	1.2	6.0	1†

* Of those chicks which cleared parasites.
† Died 35th day after inoculation.

DISCUSSION

Our data on the minimum effective dose of pyrimethamine for suppressive activity corroborate the data published by Falco *et al.* (1951). The maximum tolerated dose of the drug was 0.016 mg./gm.; the minimum effective dose, 0.00001 to 0.00002 mg./gm.; the therapeutic index was therefore about 1,000 (according to definitions and methods in Coatney *et al.*, 1953). Pyrimethamine was about 100 times as effective as chlorguanide on a dosage basis, and about 800 times as effective as quinine. In the specificity and potency of its activity against the erythrocytic forms of *P. gallinaceum* pyrimethamine is a unique antimalarial. In its prophylactic activity against sporozoite-induced infections this drug is 500 times as effective as sulfadiazine and over 1,000 times as effective

as chlorguanide. When administered in a single dose in well established sporozoite-induced infections it prevents death from tissue forms. This unique property is not possessed by any other antimalarial.

Pyrimethamine was significantly more effective against exoerythrocytic forms of the parasite than indicated by the report of Falco *et al.* (1951). These investigators reported that the drug administered prophylactically at a dosage of 2 mg./kg., while able to prevent the development of tissue forms, prolonged the life of only one of six treated chicks over the untreated controls. In our tests, employing a similar treatment regimen, the drug significantly prolonged the lives of chicks at a dosage of 0.03 mg./kg. and completely aborted the infection at 0.25 mg./kg. The only apparent difference in the techniques of the two laboratories is that Falco and her colleagues inoculated the sporozoites intravenously, while we inoculated them intramuscularly. Our results are in accord with those of Jaswant Singh *et al.* (1952).

Treatment with single doses of pyrimethamine was undertaken to determine the period over which the drug exerted its effect and the rate of elimination of antimalarial activity after administration. Comparing the data in Table 1 with those in Table 2, it is apparent that in terms of third day parasitemia and the number of days the drug exerted its effect, a dosage of 0.002 mg./gm. administered the day before inoculation of parasites was roughly equivalent to 0.001 mg./gm. administered on the day of inoculation. From a similar comparison for all dosage regimens it can be concluded that about half the antimalarial activity present on one day is lost by the next, and that residence of the drug or biologically active metabolites in the body can account for only a small fraction of the period of parasite inhibition observed when 0.002 mg./gm. was administered on the day of inoculation.

It is obvious from Tables 3, 4, and 5 that pyrimethamine has a lethal effect on tissue forms of *P. gallinaceum*. In fact, the order of activity against tissue forms is about the same as that against the erythrocytic forms of the parasite. There is one notable difference in the action of the drug against the two forms of the parasite. The compound is able to eradicate all pre-erythrocytic parasites, sterilizing sporozoite-induced infections and late exoerythrocytic parasites, but is unable to eradicate an established erythrocytic infection. Furthermore, the drug appears to have a different effect against pre-erythrocytic parasites as contrasted with late exoerythrocytic parasites. Of the 55 chicks treated prophylactically (Tables 3 and 4), 21 exhibited no infection, indicating that the pre-erythrocytic parasites had been eradicated. Of the 29 chicks which developed infections, 14 died with exoerythrocytic parasites. This suggests that in these latter chicks some of the pre-erythrocytic parasites had escaped the lethal action of the drug and were able to initiate parasitemia, and that in almost half of the chicks the tissue forms had proceeded to multiply to the point where they contributed to the death of the host. In contrast, only 1 of the 35 chicks treated on the seventh day after the inoculation of sporozoites, when presumably exoerythrocytic parasites were approaching a peak in numbers, died of an exoerythrocytic infection. It would appear, then, that pyrimethamine is more effective in suppressing the late tissue forms than the early forms. To explain this difference,

it is suggested that there is a lag of 48 hours before sporozoites develop to a stage which can be inhibited by the drug. During this period approximately 75 per cent of the drug administered has been eliminated. If this explanation should prove to be true, one would expect that treatment on days 2, 3 or 4 would be more effective in preventing death from tissue forms than treatment on the day of inoculation of sporozoites.

SUMMARY

The effect of single and multiple doses of pyrimethamine (Daraprim) against blood- and sporozoite-induced *Plasmodium gallinaceum* infections in chicks was examined. When administered twice daily for 4 days, the maximum tolerated dose for the chick was 0.016 mg./gm.; the minimum effective dose, 0.00002 mg./gm.; the drug was completely prophylactic at 0.00025 mg./gm. and partially prophylactic at 0.00003 mg./gm. When administered as a single dose, the minimum effective dose was 0.00025 mg./gm.; the completely prophylactic dose, 0.004 mg./gm.; and the partially prophylactic dose, 0.0005 mg./gm. When chicks were pretreated with single doses of pyrimethamine, about 50 per cent of the antimalarial activity disappeared per day. Single doses of 0.005 mg./gm. given to chicks with well established sporozoite-induced infections prevented deaths from tissue forms, but doses as high as 0.016 mg./gm. did not cure the infection.

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STUDIES IN HUMAN MALARIA

XXXII. The Protective and Therapeutic Effects of Pyrimethamine (Daraprim) Against Chesson Strain *Vivax* Malaria[1]

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Archibald (1951) was the first to try pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) against natural infections of malaria. He gave single oral doses of 5, 10, and 25 mg. and found that against *Plasmodium falciparum* and *P. malariae* 5 mg. "was quite as effective as many of the larger doses" in bringing about the removal of asexual parasites but the action was rather slow; gametocytes were apparently not affected.

Vincke (1952) tested pyrimethamine as a suppressant in ten isolated villages in the Belgian Congo. Each inhabitant received a single 25-mg. dose weekly for 25 weeks. At the start of the experiment 22 per cent of the population harbored malaria parasites, but at the fifteenth week no parasites could be found. The drug was not only effective as a suppressant but malaria transmission was virtually stopped as shown by the lack of sporozoites in the salivary glands of wild-caught mosquitoes.

McGregor and Smith (1952) employed pyrimethamine for treatment of frank attacks of malaria caused by *falciparum* and *malariae* parasites. At the dosages employed, 0.25 and 0.5 mg./kg., the parasites were removed from the circulating blood but the speed of action was about midway between chloroquine and chlorguanide. The fact that pyrimethamine was tasteless made it easy to administer to children.

Schneider *et al.* (1952) employed the drug therapeutically in single doses of 50 to 100 mg. or in 2 doses of 50 mg. each against infections caused by *P. falciparum* and *P. vivax* and decided that the drug was as effective as chloroquine. Other trials are reported by Field and his associates in Malaya, Gilroy and Norman in Assam, and Hay Arthur in West Bengal (See Goodwin, 1952). The workers in Malaya were dissatisfied with pyrimethamine because it acted slowly, especially against *P. falciparum*. They encountered no cross resistance between pyrimethamine and chlorguanide. Results in Assam and West Bengal were better, "fever and parasitemia were quickly controlled in most cases," this being especially true in some patients infected with *P. vivax*. Recently Bruce-Chwatt and Archibald (1953) reported on therapeutic trials using four quinine-successor drugs, one of which was pyrimethamine, and concluded that against subclinical natural infections of *P. falciparum* and *P. malariae* each of the four antimalarials proved to be good schizonticides.

[1] The work reported here and in the two papers that follow was conducted under a contract between the Office of the Surgeon General, Department of the Army, and the National Institutes of Health.

Foy and Kondi (1952) showed, in one patient infected with *P. falciparum* and treated with pyrimethamine, that gametocytes failed to develop in mosquitoes fed up to a week after the last dose of drug. Young (1952) carried out similar studies on 4 patients infected with *P. vivax* and found that mosquitoes fed before administration of a single 25-mg. dose of the drug produced infections of 45 to 100 per cent, but mosquitoes from the same lot fed 24 hours later failed to become infected. In one of these patients, mosquitoes were allowed to feed 4 hours before treatment and at 2, 4, 8, 12, 16, 20, and 24 hours after the drug was administered. Feedings four hours before drug administration gave a 90 per cent infection rate, then 55, 45, 15, and 10 per cent; the last 3 feedings were negative.

Schmidt and Genther (1953) carried out a comprehensive study of the effects of pyrimethamine against *P. cynomolgi* in monkeys. They reported on its slowness of action and forecast, as had Goodwin (1952), that probably the greatest usefulness of the drug would be in the field of suppression.

The controlled studies reported here and in the two papers that follow involved a total of 182 prisoner volunteers. The work was directed toward an appraisal of the general usefulness of pyrimethamine as an antimalarial agent. This paper reports on the suppressive, prophylactic, and therapeutic efficacy of the drug against Chesson strain *vivax* malaria.

MATERIALS AND METHODS

The subjects were white male volunteers in the U. S. Penitentiary, Atlanta, Georgia. Those selected as participants had no previous history of malaria. The methods used have been reported in an earlier paper by Coatney *et al.* (1948). Malaria was experimentally induced by the bites of infected *Anopheles quadrimaculatus* mosquitoes or by the intravenous inoculation of infected blood. In the first instance, each group of three volunteers was bitten by 10 infected mosquitoes (one group was bitten by a total of 25 infected mosquitoes on 3 nonsuccessive days) and the sporozoite densities determined by postprandial examination of the mosquitoes' salivary glands. The sporozoite density was graded 1+ to 4+; the average total inoculum (estimated by adding sporozoite densities of the mosquitoes) was never less than 22+ and generally more than 30+. The day of bite is designated as day 0. Daily blood smears were done on all subjects for the first 2 to 3 weeks, beginning on the eighth day after the bite. They were then made 2 to 3 times weekly for the following few weeks and then once weekly until the end of the observation period. When relapses occurred, smears were made daily until parasites were no longer present in the circulating blood. White blood cell counts, methemoglobin determinations, hematocrit measurements and urinalyses were done at regular intervals as part of the follow-up studies. All subjects were observed for at least six months and some for over two years.

Treatment of frank attacks was started on the third day of patent parasitemia, provided there had been an oral temperature of 101°F. or more, otherwise treatment was withheld until the fourth or fifth day. All treatments were given orally. Unless otherwise stated, all dosages were in terms of the base.

TESTS AND RESULTS

I. *Protective Trial*

Suppression. Our first trial was designed to test for the suppressive action of pyrimethamine with chloroquine and chlorguanide as drugs of reference. In earlier studies of this series (Lints *et al.*, 1950) each of the latter two drugs had effectively suppressed this strain of *vivax* for 6 or for 12 months when given in single weekly doses of 300 mg.

Thirty-six volunteers were infected by the bites of 10 heavily infected mosquitoes on 13 July 1951. Twelve of these men were divided into 3 groups of 4 men each and the remainder served as controls. The first group received 25 mg. of pyrimethamine, the second group received 300 mg. of chlorguanide (salt), and the third group received 300 mg. of chloroquine. The 12 test subjects received 17 weekly doses of drug beginning on day 0. As shown in Table 1, none of the subjects in the treated groups showed patent parasitemia during the period of drug

TABLE 1

The suppressive effect of weekly doses of pyrimethamine against sporozoite-induced Chesson strain vivax *malaria*

NO. VOLUNTEERS	DRUG REGIMEN	NO. DOSES	COMPLETE SUPPRESSION	DAYS FROM LAST TREATMENT TO FIRST + BLOOD SMEAR
4	Pyrimethamine, 25 mg.	17	Yes	515,
4	Chlorguanide, 300 mg.	17	Yes	10, 16, 17, 17
4	Chloroquine, 300 mg.	17	Yes	32, 34, 38, 42
20	Controls, no drug	Patent 10 to 13 days after bite		

administration, while the controls came down with malaria 10 to 13 days after the bites. The pyrimethamine-treated subjects all remained negative for approximately 17 months after the last dose of drug and then, on day 515, volunteer AP-103 came down with fever and parasitemia. The other patients in this group are still negative 20 months after the last dose of drug.

When it became evident that a single 25-mg. dose per patient per week for 17 weeks was not only suppressive but appeared to eradicate the infection, it was decided to limit the administration of the drug to 8 weekly doses and to determine the minimal suppressive dosage. With the exception of the first two trials in this series of tests, the drug was started 6 days before the bite (0–6) and continued at weekly intervals for 8 doses. This modification was made to see if changing the time of administration relative to the bite would alter the results. The results are shown in Table 2 where it can be seen that all dosages down to and including 0.8 mg. were completely suppressive; breakthroughs occurred in those given 0.4 mg. The subjects bitten by 10 infected mosquitoes and given 25 mg. per week have remained free of infection to date, approximately 12 months after the last dose of drug. The group which experienced bites on three separate

occasions and received 25 mg. per week were parasite free during treatment, but two developed malaria after the drug was discontinued.

Prophylaxis. The fact that the pyrimethamine-treated subjects in the first protective trials (Table 1) had failed to come down after the drug had been stopped for several months suggested the possibility that pyrimethamine might be acting as a true causal prophylactic. To test for this mode of action 6 volunteers were bitten by 10 infected mosquitoes, and directly thereafter 3 of the volunteers were each given a single 25-mg. dose of pyrimethamine. The other three subjects acted as controls and received no drug. The 3 treated subjects came down with malaria, but not until 72, 72, and 142 days, respectively, after the bites, showing that although the drug was not a true causal prophylactic under the conditions of our test, it did bring about a pronounced delay in patent parasitemia, as the controls came down in 13 to 14 days.

TABLE 2

Suppressive effect of weekly doses of pyrimethamine against sporozoite-induced Chesson strain vivax *malaria*

DRUG DOSAGES	NO. VOL.	INOCULUM + MOSQ.	NO. DOSES	DRUG REGIMEN	COMPLETE SUPPRESSION	DAYS FROM LAST TREATMENT TO 1ST + BLOOD SMEAR	CONTROLS POSITIVE	
							No. Vol.	Days after bite
mg.								
25.0	3	10	8	0 + 49	Yes		3	13–14
25.0	3	25*	8	0 + 49	Yes	39, 67,	4	10–12
12.5	3	10	8	−6 + 43	Yes	41, 46, 52	6	12–13
6.25	3	10	8	−6 + 43	Yes	29, 31, 48		
3.1	3	10	8	−6 + 43	Yes	22, 38, 40	4	10–12
1.6	3	10	8	−6 + 43	Yes	21, 22, 24		
0.8	3	10	8	−6 + 43	Yes	10, 21, 30		
0.4	3	10	8	−6 + 43	Breakthrough day 10, 18, 42, after day 0		3	12–13

* Subjects bitten by mosquitoes on 3 occasions 2 days apart.

Effect of drug given some days prior to infection. Therapeutic studies, given in detail in another section, seemed to indicate that the drug or an active metabolite persisted in the body for considerable time following drug administration, since relapses seldom occurred in the first 40 days after treatment. Because no chemical method exists for measuring the concentrations of pyrimethamine in man following the administration of single small doses of the compound, it was decided to test for drug persistence biologically. The first test for persistence was set up to measure activity against sporozoites or their immediate offspring. The plan was to have volunteers bitten by infective mosquitoes at increasing time intervals after receiving a single 25-mg. dose of the drug. To accomplish this ten men were divided into pairs; the first pair received the single dose 37 days before it was planned to have infective mosquitoes available. The second pair was given the same treatment 7 days later and so on until the last pair received the drug 9 days before the whole group was exposed simultaneously to infective bites by

mosquitoes. As shown in Table 3, 12 men took part in the test (an institutional transfer caused the loss of one man from the study) and were bitten by heavily infected *Anopheles quadrimaculatus* mosquitoes on day zero (21 November 1952). The controls came down with malaria in 13 to 14 days and all but 2 of the treated subjects exhibited patent parasitemia 12 to 29 days after infection; of these two, one developed parasitemia after 122 days, the other is still negative 7 months later. The delay of patent infection in AP-339 and the failure of AP-341 to develop malaria presumably were due to the effect of the drug, because each of these subjects was bitten second in a three-man biting sequence; the first and third man in each group came down on day 13 or 14. As further evidence of drug action volunteer AP-342 did not show patent parasitemia until day 29. This late appearance of parasites might have been due to the suppressive effect of the drug or to a partial prophylactic effect which was complete in AP-341.

TABLE 3

Effect of giving a single 25-mg. dose of pyrimethamine at various intervals prior to infection by mosquito bite

VOL. NO.	DRUG DAYS BEFORE BITE					PRE-PATENT PERIOD (DAYS)
	37	30	23	16	9	
AP-334	X					14
AP-335	X					12
AP-336		X				14
AP-337			X			16
AP-338			X			16
AP-339				X		122
AP-340				X		14
AP-341					X	Negative 201 days
AP-342					X	29
AP-343	No drug					13
AP-344	No drug					14
AP-345	No drug					14

The second test for persistence of drug in the body was set up to measure activity of pyrimethamine against parasites in circulating blood. Fifteen volunteers were given the drug and sixteen served as controls. As shown in Table 4, the treated subjects were given the drug 3 to 17 days before each of the 31 participants received 1,000,000 parasites intravenously on the same day from a single donor whose parasites had never been exposed to pyrimethamine. The treated subjects have shown no evidence of infection during 7 months of observation. All of the controls were parasite-positive 4 days after inoculation.

II. *Therapeutic Trials*

Single dose treatment. During the course of these studies there was opportunity to treat 30 attacks of malaria in 24 individuals in whom the infection had been induced by sporozoites (Table 5). Of the 26 attacks treated with a single 25-mg.

TABLE 4

Effect of giving volunteers a single 25-mg. dose of pyrimethamine at various intervals prior to intravenous inoculation with blood containing one million Chesson strain vivax *parasites*

VOL. NO.	DRUG DAYS BEFORE INOCULATION										PRE-PATENT PERIOD	SIXTEEN CONTROLS
	17	15	14	12	11	9	8	6	5	3		
AP-346	X										No evidence of infection during 7 months of observation	All patent on day 4
AP-347	X											
AP-346a		X										
AP-348			X									
AP-349			X									
AP-348a				X								
AP-350					X							
AP-351					X							
AP-350a						X						
AP-352							X					
AP-353							X					
AP-352a								X				
AP-354									X			
AP-355									X			
AP-354a										X		

TABLE 5

Therapeutic effect of single dose treatment against attacks of sporozoite-induced Chesson strain vivax *malaria*

NO. VOL.	℞ OF PRIMARY ATTACK (NO PREVIOUS SUPPRESSION)	℞ OF PRIMARY ATTACK (AFTER SUPPRESSION)	℞ OF FIRST RELAPSE	℞ OF SECOND RELAPSE	DAYS TO CLEAR PARASITES (AFTER ℞)	DAYS TO NORMAL TEMP. (AFTER ℞)	FIRST RELAPSE (DAYS AFTER ℞)	SECOND RELAPSE (DAYS AFTER ℞)	THIRD RELAPSE (DAYS AFTER ℞)
9	50–63* 25 mg.				2,3,4,4,5, 5,8,8,10	3,3,3,3,3, 3,3,4,4	48,55,†		
11		50–63 25 mg.			2,2,2,3,3, 4,5,7,7,7, 9	2,2,2,3,3, 4,4,4,4, 4,5	15,21,31, 37,48,‡		
4	§		50–63 25 mg.		3,5,6,7	2,3,3,4		43,43, 45,55	
2	‖		‖	50–63 25 mg.	2,6	0,2			53,54
2	7618¶ 600 mg.				2,2	3,4	27,31		
2		7618 600 mg.			3,3	2,2	38,39		

* Pyrimethamine.

† Seven men started on suppression before first relapse.

‡ Six men started on suppression before first relapse.

§ Primary attack also treated with 50–63 single dose 25 mg.

‖ Primary attack and first relapse treated with 50–63 single dose 25 mg.

¶ Chloroquine.

dose of pyrimethamine, 9 were early primaries, 11 were primary attacks following suppression, 4 were first relapses, and 2 were second relapses. Four attacks were treated with single 600-mg. doses of chloroquine; 2 were early primary attacks and 2 were primary attacks following suppression. Among the individuals treated with pyrimethamine, clinical improvement was evident within 24 to 36 hours. The temperature gradually returned to normal in one to four days, mean 3.0 days; the blood was cleared of parasites in 2 to 10 days, mean 5.0 days. In this limited number of cases there were no significant differences in the response to pyrimethamine therapy whether the attack was an early primary, a delayed primary, or a relapse. There were 13 relapses in this group (the 13 other patients were placed on suppression before the first relapse had time to occur) which occurred 15 to 55 days (mean 42 days) after therapy. In 4 similar cases treated with a single dose of chloroquine (Table 5) the temperature returned to normal in 2 days, and the blood was free of parasites after 3 days.

TABLE 6

Suppressive effect of 8 weekly 25-mg. doses of pyrimethamine begun after treatment of the initial attack. A single 25-mg. dose of pyrimethamine was used for treatment of the attack

NO. VOL.	PREVIOUS SUPPRESSION	DAYS FROM LAST TREATMENT TO 1ST + BLOOD SMEAR	AVERAGE SPOROZOITE DOSAGE (PLUSES)	OBSERVATION PERIOD IN NON-RELAPSED CASES (DAYS)
3	Yes	190,	31	557
3	Yes	36,	31	557
4	No	37, 43, 45, 45	36	
3	No		28	419

Single dose treatment followed by weekly suppressive doses. In an attempt to learn the effects of a period of suppression after therapy of the acute attack, two types of infection were used, (1) those against which pyrimethamine suppression had been employed prior to the initial attack and (2) those without any previous suppressive medication. Six volunteers made up the first group, three had developed malaria after suppression with 6.25 mg. weekly and the other three after suppression with 12.5 mg. weekly (Table 2). Seven newly-infected volunteers comprised the second group.

In all cases a 25-mg. dose of pyrimethamine was given on the third day of parasitemia for treatment of the frank attack; suppressive treatment was begun one week later, at a dosage of 25 mg., and continued until 8 weekly doses had been given. The results are shown in Table 6.

After an observation period ranging from 419 to 557 days, only 6 of the 13 patients have shown reappearance of parasites. Although the various groups involved are too small for definitive comparisons, it is noteworthy that the majority of relapses occurred in one group of 4 volunteers simultaneously infected by the same lot of mosquitoes, the most heavily infected lot used in this particular experiment. Each of these four had relapses. This suggests that sporozoite dosage

is an important factor in determining whether or not cure results from this particular drug regimen.

Single dose daily for seven days. When it appeared that cure of the infection had been obtained in 7 out of 13 individuals whose primary attack had been treated by giving a single 25-mg. dose of pyrimethamine followed by 8 weekly 25-mg. doses (Table 2), it was thought advisable to seek cure of the infection by shortening the time of drug administration to 7 daily doses. Ten early attacks were treated according to this regimen. Cure was obtained in only 3 individuals; relapses occurred in the other subjects 50 to 102 days (mean 72 days) later.

DISCUSSION

There is ample evidence in these and other studies to prove that pyrimethamine is a potent antimalarial drug. In terms of the dosage required to produce effects against Chesson strain *vivax* malaria it is the most active compound yet discovered. Its ultimate place in the treatment of malaria will depend, however, not only upon its absolute activity against erythrocytic parasites, but also upon its rapidity of action, its ability to produce permanent cure, its relative toxicity, and upon the readiness with which parasites become resistant to its action. While many of these questions can only be answered with finality by actual field trials, there is considerable evidence available upon which to base predictions.

As to the extraordinary effectiveness of the drug against erythrocytic parasites there can be no doubt. The clearance of parasites by single doses of 25 mg., the temporary clearance of trophozoite-induced infections by 0.25 mg. per day (Hernandez *et al.*, 1953), the suppressive effect of 0.8 mg. per week, and the fact that for at least 17 days after a single dose of 25 mg., one cannot initiate infections by injecting a million erythrocytic parasites, all indicate a drug with amazing ability to interfere with the development of the erythrocytic *vivax* parasite.

Evidence for action on exoerythrocytic stages is less dramatic. The drug does not appear to be a true causal prophylactic in tolerable doses, although the long delays resulting from single doses on the day of bites favor some deleterious action on the early stages of the parasite. Additional and stronger evidence for action on exoerythrocytic parasites is provided by the fact that suppressive treatment with 25 mg. of pyrimethamine given once a week for 8 weeks brought about the suppressive cure of many infections. As will be pointed out later, this suggests the most promising application of the drug in the management of malaria.

When used to treat acute attacks of Chesson strain *vivax* malaria, pyrimethamine is effective in minute doses but even relatively large doses will not clear parasites and fever as rapidly as chloroquine. Single-dose therapy was invariably followed by relapses. Treatment with 25 mg. per day for one week cured only 3 of 10 subjects. Weekly dosage for 8 weeks after single-dose treatment of the acute attack led to cures in approximately one-half of the subjects.

This latter use is analogous to suppressive use of the drug for 8 weeks with dosage being started before mosquito bites, and the results are comparable.

Although such application seems to eradicate many infections permanently, there is relatively little margin of therapeutic safety in the procedure, inasmuch as halving the dosage or increasing the infective inoculum leads to a failure to produce permanent cure or prevention of infections. This in turn would suggest that in practical application one might except widely divergent results in different groups of naturally-infected subjects, dependent upon the innate characteristics of the strain of malaria and the intensity of exposure.

The fact that erythrocytic parasites do not survive when injected into volunteers who had been given a single 25-mg. dose of pyrimethamine 17 days previously indicates not only the high degree of activity of the drug against erythrocytic parasites, but also shows that the drug or its active metabolites persist in the body for a considerable period of time. An end-point was not reached in this experiment. Similar tests against sporozoite-induced infections provided less convincing evidence of drug persistence, because action against early pre-erythrocytic stages is less clear-cut (e.g. 25 mg. on the day of bite delays, but does not prevent infection), and because temporary suppression of early erythrocytic stages could also account for the results. In any event, these tests provide additional evidence to show why weekly doses can be expected to provide a considerable margin of safety against break-throughs while drug is being taken, even if occasional doses are missed.

The toxicity of pyrimethamine has been discussed elsewhere (Schmidt *et al.*, 1953a; Myatt *et al.*, 1953). The administration of 25 mg. of the drug *daily* for several weeks may produce megaloblastic anemia, but no harmful effects have been noted in the individuals given the smaller dosage regimens reported in this paper. The anemias reported by Myatt *et al.* disappeared upon cessation of therapy, but the possibility of their occurrence should be kept in mind if long-continued administration is proposed in large populations, particularly those on suboptimal diets.

The fact that *vivax* parasites can develop resistance to pyrimethamine, as shown by Hernandez *et al.* (1953) must be seriously considered when making recommendations as to widespread use of the drug. Experience with chlorguanide has shown the likelihood of such resistance developing in actual practice, if the mode of action of a compound is such that resistance can be developed under experimental conditions.

It is of interest to note in how many respects the results of these tests using the Chesson strain of *P. vivax* agree with those obtained by Schmidt and Genther (1953) in their evaluation of pyrimethamine against *P. cynomolgi* infections in the rhesus monkey. Against both species of parasite prophylactic use led to prolonged delays in the incubation period but no permanent prevention of infection; there was remarkable potency against erythrocytic parasites, but a flat dosage response curve and relatively slow clearance of parasites; in both instances drug resistance was readily acquired. Schmidt and Genther were unable to obtain permanent cures in any sporozoite-induced infections, but they did not report on the type of regimen we found most effective, i.e. weekly dosage for 8 or more weeks, in dosages comparable with those we employed. Their regimen

corresponding most nearly to this was 0.15 mg./kg. (roughly 10 to 12 mg. in an average human patient) for 6 weeks begun just prior to mosquito bites which did not produce any cures. Schmidt and Genther predicted however, on the basis of their observations that pyrimethamine injures the exoerythrocytic stages of simian malaria, that prophylaxis or cure would be achieved occasionally in *vivax* infections.

In conclusion, pyrimethamine does not appear to be as good as chloroquine in the treatment of acute attacks of malaria, despite the fact that if several doses are given, the chances of permanent cure of *vivax* infections are increased. It has, however, many properties of an admirable suppressive agent. Acute attacks are prevented while the drug is being taken, and, in contrast with chloroquine and chlorguanide, suppression for 8 to 17 weeks is followed by relatively few delayed attacks. Careful field studies are warranted. In addition, studies as to the mode of action of pyrimethamine are highly desirable; its extraordinary potency should provide important clues to the metabolism of malarial parasites.

SUMMARY

This study was undertaken to evaluate the protective and therapeutic efficacy of pyrimethamine (Daraprim) against Chesson strain *Plasmodium vivax* in human volunteers. The drug was highly effective as a suppressive agent, 0.8 to 25 mg. gave complete protection during drug administration; it was less efficient when used for therapy of acute attacks. Pyrimethamine does not appear to be a true causal prophylactic although prolonged delays before patent parasitemia suggested some deleterious effect against early tissue stages of the parasite. Weekly administration of the drug for 8 or more weeks resulted in suppressive cure of many infections. Probably the greatest usefulness of the drug will be for routine suppression.

Acknowledgment

We wish to make special acknowledgment and extend thanks to Mr. James V. Bennett, Director of the Federal Bureau of Prisons, to Mr. William H. Hiatt, Warden of the U. S. Penitentiary, Atlanta, Georgia, to Dr. H. M. Janney, Chief Medical Officer of that institution and their respective staffs, whose interest and active cooperation made these studies possible.

We also extend thanks to the Medical Service of the Army for providing some of the laboratory and nursing personnel who contributed so much to the over-all operation of the project, and to Dr. George Hitchings of the Burroughs Wellcome Laboratories, Tuckahoe, N. Y., for supplying the pyrimethamine for these studies.

It is difficult for us to make proper acknowledgment to the inmates who volunteered for this project. These men willingly took the drug, bore the discomforts of malaria, and submitted to the necessary laboratory procedures. We extend our thanks to them.

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STUDIES IN HUMAN MALARIA

XXXIII. The Toxicity of Pyrimethamine (Daraprim) in Man

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Pyrimethamine and similar 2,4-diaminopyrimidine compounds were originally developed as antimetabolites by Hitchings *et al.* (1950, 1950a), Hitchings (1952). These compounds were tested for antimalarial activity when the recognition of an analogy between the structural formula of one of them (2,4-diamino-5-*p*-chlorophenoxy-pyrimidine) and that of chlorguanide (N_1-(*p*-chlorophenyl)-N_5-iso-propylbiguanide) was noted (Falco *et al.*, 1949). In subsequent trials pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) proved to have the greatest antimalarial activity in this group of 2,4-diaminopyrimidine compounds (Falco *et al.*, 1951; Rollo, 1952).

Toxicity studies have been carried out in dogs by Hamilton (1952) and in rats and monkeys by Schmidt *et al.* (1953). In man studies of the toxicity were somewhat limited in scope. Goodwin (1952) himself took 50 mg. of pyrimethamine twice weekly for approximately 10 weeks, then 25 mg. daily for 51 days, then 5 mg. daily for a period of about 9 months. Additional doses of 50 mg. were taken on three occasions, 25 mg. on two occasions, and 100 mg. on two occasions during the test period. No toxic reactions of any kind were noted. Blood counts were normal and the sternal marrow was normal when examined at the end of the test period. In another test (Goodwin, 1952) 13 volunteers took 50 mg. twice a week for 3 months. Two developed slight gastrointestinal upsets, but no changes occurred in red cell count, white cell count, sedimentation rate, or in the urine.

Jones (1952) gave pyrimethamine to 11 patients with incurable diseases. The dosages were 300 mg. daily for 6 to 8 days in 2 patients, 200 mg. daily for 11 days in 3 patients, and 100 mg. daily for 16 to 18 days in the remaining 6 patients. All experienced anorexia, abdominal pain, nausea and vomiting within a few days, which necessitated stopping the drug.

Because of the pronounced effectiveness of this drug as an antimalarial it was considered advisable to carry out detailed studies of its toxicity in volunteers under controlled conditions. The studies we are reporting here were carried out at the U. S. Penitentiary, Atlanta, Georgia

MATERIAL AND METHODS

Twelve white male volunteers were used. These men were from 23 to 36 years of age and varied in weight from 128 lbs. (59 kg.) to 203 lbs. (92 kg.). History and physical examinations showed them to be in excellent health.

Each volunteer was given 25 mg. of pyrimethamine daily, starting on May 23, 1952, and continuing through July 9, 1952, in one case, and through July 10, 1952, in the remaining 11 cases. This represented a total of 48 daily doses for one

volunteer and 49 daily doses for 11. On a mg./kg. basis the daily dosage ranged from 0.27 mg. to 0.43 mg./kg.

Prior to starting the drug and at intervals during and after the administration period complete histories and physical examinations were done in addition to the following laboratory studies: electrocardiogram, red cell count, hemoglobin, methemoglobin, hematocrit, white cell count, differential white cell count, eosinophile count, blood nonprotein nitrogen, serum thymol turbidity, bromsulfalein retention, urinalysis, urine urobilinogen, urine concentration and dilution tests, Addis count of urinary sediment, and endogenous creatinine clearance. In addition, sternal marrow aspirations were done on 10 of the 12 volunteers 3 days after discontinuation of the drug and again on 3 of the original 10 at 50 days after the drug was stopped.

TABLE 1

Effect of pyrimethamine (Daraprim), 25 mg. single dose daily for 7 weeks, on hemoglobin (gm. per 100 cc.) in 12 volunteers

VOL. NO.	4 WKS. BEFORE TEST	START OF TEST	WEEKS OF TEST																		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
			Daraprim 25 mg. daily																			
1	14	15	14	13	14	13	14	13	13	13	13	13	14	14	14	—	—	14	14	—	14	
2	14	16	17	15	14	15	15	13	15	14	15	15	16	16	16	16	—	16	16	—	16	
3	14	13	13	13	12	13	14	11	13	12	12	12	12	12	13	13	—	13	13	—	13	
4	13	14	14	14	13	13	14	13	12	12	12	12	13	12	13	13	—	14	14	—	15	
5	14	14	15	14	13	12	14	11	10	10	12	13	14	14	14	14	—	14	16	—	16	
6	16	14	15	15	15	17	15	16	16	16	14	15	16	16	15	15	—	16	16	—	16	
7	15	15	16	15	14	14	13	13	13	13	13	13	15	15	14	15	—	15	16	—	15	
8	16	15	16	16	15	14	15	14	15	15	15	14	15	15	15	17	—	16	16	—	16	
9	15	15	15	15	15	14	14	14	14	15	14	14	15	14	15	14	—	16	16	—	15	
10	14	15	14	15	15	15	15	14	14	13	14	14	15	15	14	14	—	15	14	—	15	
11	14	15	16	16	15	16	15	15	15	14	15	14	16	15	15	15	—	14	16	—	16	
12	17	15	16	15	16	15	15	15	14	12	15	14	15	15	14	15	—	15	16	—	16	

RESULTS

One man, who became anemic, developed pallor, malaise and weakness beginning about the thirtieth day of the test. This condition continued until the sixtieth day. No other symptoms appeared in any of the volunteers and all of the men continued to work at their regular jobs in the institution.

Three men, who became mildly anemic, developed increased capillary fragility as measured by the tourniquet test on the forty-fifth day of drug administration. In one of these men spontaneous petechiae occurred on the left arm and right leg on the forty-fifth day. No other objective physical findings appeared.

There were decreases in the red cell count, hemoglobin and hematocrit of six volunteers about the thirtieth day which persisted as long as the drug was given (Tables 1, 2, 3, and 4). These values promptly returned to normal after discontinuation of the drug. Table 1 shows the hemoglobin values for each volunteer one month before and at regular intervals during the test period. The greatest drop in hemoglobin occurred in volunteer No. 5; his hemoglobin level dropped to

10.0 grams on July 9, 1952, the forty-eighth day of drug administration. During the test period this same volunteer showed no free acid in the gastric juice after histamine. Two months after stopping the drug free acid was present, but at a low level. Other volunteers who developed a drop in hemoglobin had normal gastric acidity values. Hemoglobin values of the four men who became most

TABLE 2

Effect of pyrimethamine (Daraprim), 25 mg. single dose daily for 7 weeks, on red blood cell count (M per cu. mm.) in 12 volunteers

VOL. NO.	4 WKS. BEFORE TEST	WEEKS OF TEST																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	28
		Daraprim 25 mg. daily																			
1	4.6	4.8	4.3	4.2	4.0	4.0	4.2	4.2	4.0	4.0	4.0	4.4	4.1	4.8	—	—	—	4.8	—	4.5	
2	4.7	4.9	4.5	4.3	4.3	4.1	4.5	4.5	4.7	4.1	3.8	4.6	4.5	5.1	4.5	—	—	5.4	—	5.3	
3	4.2	4.2	4.1	3.9	3.7	4.0	4.5	4.1	4.3	3.6	3.9	3.5	4.0	3.8	4.0	—	—	3.9	—	4.1	
4	4.1	4.0	3.9	4.1	3.6	4.1	5.1	4.3	4.0	3.9	3.6	3.7	3.9	3.2	4.0	—	—	4.4	—	4.7	
5	5.7	4.6	4.4	3.9	3.9	3.8	3.7	3.9	3.6	3.3	3.5	4.2	4.0	4.2	4.6	—	—	5.3	—	5.0	
6	5.1	4.4	4.4	4.3	4.5	4.3	5.2	5.3	4.4	4.1	4.6	4.4	4.3	4.5	3.8	—	—	5.0	—	5.3	
7	4.8	4.2	4.0	4.3	4.3	4.3	5.1	4.1	4.3	4.2	4.0	3.6	4.5	5.6	4.4	—	—	5.1	—	5.1	
8	5.0	4.6	5.4	4.3	4.1	4.0	4.3	5.3	5.0	4.4	4.0	4.3	4.5	4.6	5.1	—	—	5.0	—	5.2	
9	5.5	4.4	5.5	3.9	4.3	4.0	4.5	4.8	4.6	4.1	3.8	5.0	4.0	5.0	4.2	—	—	4.9	—	4.9	
10	5.4	4.8	5.2	4.5	4.1	4.3	4.7	5.2	4.9	4.8	4.8	4.8	4.5	4.9	4.6	—	—	4.8	—	5.1	
11	4.8	4.7	5.4	4.9	4.8	4.5	5.4	5.1	4.7	4.7	4.3	4.7	4.2	—	4.3	—	—	5.1	—	5.0	
12	5.3	4.2	5.2	4.6	4.0	4.0	5.0	4.4	4.5	4.0	4.5	4.3	3.8	4.6	5.4	—	—	5.0	—	5.0	

TABLE 3

Effect of pyrimethamine (Daraprim), 25 mg. single dose daily for 7 weeks, on hematocrit (per cent) in 12 volunteers

VOL. NO.	1 WK. BEFORE TEST	WEEKS OF TEST																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
		Daraprim 25 mg. daily																		
1	49	—	43	42	41	39	44	47	41	44	44	46	44	48	—	—	—	47	—	46
2	51	—	51	48	49	47	47	47	47	50	52	53	53	53	55	—	—	54	—	54
3	49	—	43	41	40	42	37	43	40	40	41	41	40	43	44	—	—	43	—	42
4	46	—	45	41	41	41	41	40	39	38	38	42	41	43	44	—	—	47	—	48
5	49	—	43	41	40	39	35	32	31	38	42	46	44	47	50	—	—	52	—	49
6	51	—	51	49	52	49	53	49	53	48	51	52	53	49	52	—	—	52	—	52
7	49	—	49	46	44	42	43	41	41	43	45	49	51	47	52	—	—	52	—	52
8	48	—	52	51	46	48	46	50	51	49	49	51	51	50	56	—	—	52	—	51
9	49	—	48	49	47	44	46	44	46	45	46	49	45	48	47	—	—	51	—	50
10	40	—	50	50	49	50	47	48	48	47	50	48	50	47	50	—	—	49	—	50
11	49	—	53	51	52	48	50	49	50	49	49	52	51	51	51	—	—	53	—	51
12	53	—	49	51	48	46	47	46	47	47	47	49	48	48	53	—	—	51	—	51

anemic are shown graphically in Figure 1. The hemoglobin values promptly returned to normal when the drug was discontinued. The values for the red blood counts and hematocrits are given in Tables 3 and 4.

Sternal marrow changes of the megaloblastic type involving both the erythroid and myeloid elements occurred in six volunteers. The subjects showing the greatest megaloblastic changes were those with the severest anemia (Plate I,

TABLE 4

Effect of pyrimethamine (Daraprim), 25 mg. single dose daily for 7 weeks, on bone marrow in 12 volunteers

VOL. NO.	WEIGHT (LBS.)	PER CENT DECREASE HEMOGLOBIN	PER CENT DECREASE HEMATOCRIT	BONE MARROW STUDY	
				3 days after discontinuation of drug	50 days after drug
1	157	8	6	Not done	Not done
2	166	7	8	Normal	Not done
3	155	8	18	Moderate changes of the "pernicious anemia type" in erythrocytes and granulocytes	Normal
4	130	14	17	Mild changes of the "pernicious anemia type" in erythrocytes and granulocytes	Normal
5	128	29	35	Marked changes of the "pernicious anemia type", especially in granulocytes	Normal
6	203	0	0	Hypersegmentation of neutrophiles	Not done
7	150	15	16	Moderate changes of the "pernicious anemia type" in erythrocytes and granulocytes	Not done
8	170	5	0	Normal	Not done
9	136	7	8	Not done	Not done
10	203	7	4	Normal	Not done
11	187	0	0	Normal	Not done
12	140	19	11	Moderate changes of the "pernicious anemia type" in granulocytes	Not done

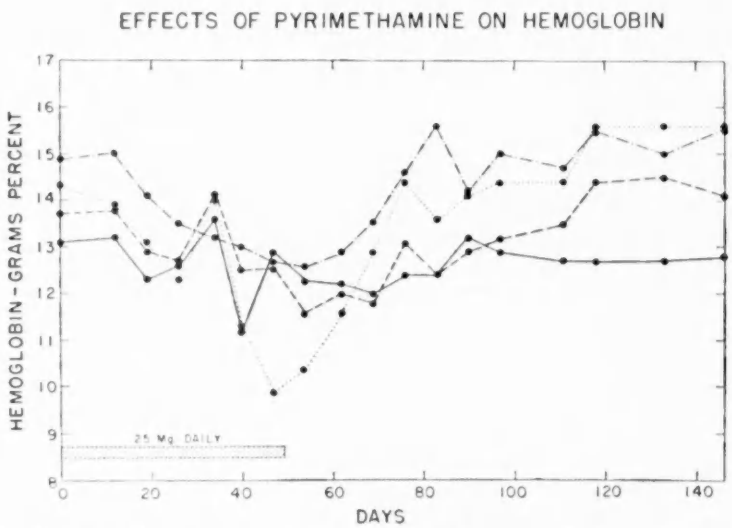

FIG. 1. Hemoglobin values (gram per cent) of four patients while receiving and after receiving Daraprim 25 mg. daily.

Figures 1, 2, 3, and 4). Also, the smaller men showed the greatest changes in the sternal marrow and in the peripheral blood (Table 4). A second sternal aspiration in subjects Nos. 3, 4, and 5 done 50 days after the drug was stopped gave a normal histological picture. All changes were reversible without specific medication.

During the test the peripheral white cell count and differential white cell count remained normal at all times. Total eosinophile counts remained within normal limits and the kidney and liver function tests indicated no disturbance of function. At the conclusion of the drug administration period prothrombin times, bleeding times and clotting times were normal in all volunteers.

DISCUSSION

In this study six of twelve human volunteers given 25 mg. of pyrimethamine daily for 49 days developed mild to moderate anemia of the macrocytic normochromic type. The bone marrow picture in the men developing anemia was of the megaloblastic type, similar to the type seen in folic acid deficiency and "extrinsic factor" deficiency anemias. The anemias disappeared and the bone marrow changes promptly returned to normal when the drug was discontinued.

Hamilton *et al.* (1952) produced a megaloblastic bone marrow in dogs using toxic doses of pyrimethamine. Apparently the results were similar to those we have obtained in man.

Schmidt *et al.* (1953) found that toxic doses of pyrimethamine (2.5 mg./kg. to 40 mg./kg.) given to rats and to monkeys produced profound decreases in the lymphocytes and polymorphonuclear cells of the peripheral blood with little effect on the erythrocytes. Our results showed the reverse, namely, no effect on lymphocytes or polymorphonuclear cells but a mild to moderate decrease in the number of erythrocytes. Schmidt also found that in monkeys pyrimethamine produced a bone marrow showing marked changes in the myeloid cells and lesser changes in the erythroid series. In man, the bone marrow exhibited changes in both the myeloid and erythroid series consisting mainly of a shift in size and morphology. Atrophy of the spleen and lymphatic tissues as occurred in monkeys was not evidenced in man although the possibility of some damage cannot be entirely excluded because no lymph node biopsies were done. In monkeys, kidney lesions were found in animals dying of pyrimethamine toxicity. The lesions consisted of irregular enlargement of the lining epithelium of the distal part of the calyx and other non-specific changes in glomeruli and tubules. In man, no change in kidney function could be detected by available tests during or after the administration of pyrimethamine.

Differences in response to pyrimethamine by man and by monkeys can probably be accounted for on the basis of species differences. Two of Schmidt's monkeys given 1.25 mg./kg. for 42 days showed no outward signs of toxicity, nor did two of four on dosage of 2.5 mg./kg. In man, however, dosages of 0.27 mg./kg. to 0.43 mg./kg. for 49 days produced toxic changes. This might be taken to indicate that pyrimethamine is more toxic to man than to monkeys. On the other hand, while toxicity may be produced at lower dosage in man, the toxic reactions are much less severe than in monkeys.

In other studies employing pyrimethamine at various dosages against *vivax* malaria (Coatney *et al.*, 1953) no toxic reactions were produced. Four subjects received 25 mg. weekly for 17 weeks and nineteen others 25 mg. for 8 weeks without any ill effects. The same was true of fourteen subjects who received 25 mg. daily for 7 days and seven others who received 12.8 mg. daily for 7 days.

Mechanisms by which pyrimethamine produces toxicity in man are not clearly known at this time. Apparently it is a matter of dosage since the smaller men in our series tended to show more changes than the larger ones, though admittedly a larger series might not bear this out. The changes produced are similar to those seen in folic acid deficiency anemia, but before one can say that the anemia produced is due to the anti-folic and anti-folinic action of pyrimethamine, further studies will need to be done in order to show a reversal of the anemia by folinic acid while continuing the administration of the drug and protection to toxic doses of pyrimethamine by giving concurrent folinic acid.

There has been no evidence of individual idiosyncrasy to pyrimethamine during our studies with the drug, which we have now given to 175 men.

Our studies show that there is an ample margin of safety for the use of pyrimethamine in man. A single dose of 25 mg. will eradicate the erythrocytic parasites of *Plasmodium vivax* and will suppress the tissue stages for over a month. For use as a suppressant the drug need not be given more than once a week and in doses of no more than 25 mg. Barring rare cases of idiosyncrasy no toxicity with this weekly dosage is likely.

Although our studies show toxicity at doses of 25 mg. daily and not at 25 mg. weekly, the margin of safety is much more than the apparent seven times.

SUMMARY

Pyrimethamine (Daraprim) was given to 12 healthy volunteers for 49 days at a dosage of 25 mg. single dose daily. Six subjects developed a megaloblastic type of anemia with rapid remission when the drug was stopped.

Acknowledgments

We wish to express our appreciation to our consultant in internal medicine, Dr. David James, Associate Professor of Medicine, Emory University School of Medicine, who cooperated in the management of the patients; to our consultant hematologist, Dr. Charles M. Huguley, Jr., Assistant Professor of Medicine, Emory University School of Medicine, who examined and interpreted all the sternal marrow smears and made the color photographs; to Drs. James A. Shannon and Robert W. Berliner of the National Institutes of Health for their help and advice during the planning and execution of these studies.

Special acknowledgment and thanks are extended to Mr. James V. Bennett, Director of the Federal Bureau of Prisons; to Mr. William H. Hiatt, Warden of the U. S. Penitentiary, Atlanta, Georgia; to Dr. H. M. Janney, Chief Medical Officer of that institution; and to their respective staffs who made these studies possible.

Thanks are also extended to the Medical Service of the Army for providing some of the laboratory and nursing personnel who contributed so much to the operation of the over-all project and to Dr. George Hitchings of the Burroughs Wellcome Laboratories, Tuckahoe, N. Y., for supplying the pyrimethamine used in these studies.

We cannot overemphasize the contribution made by the inmate volunteers who willingly took the experimental drug. We extend our thanks to them for their contribution to this study.

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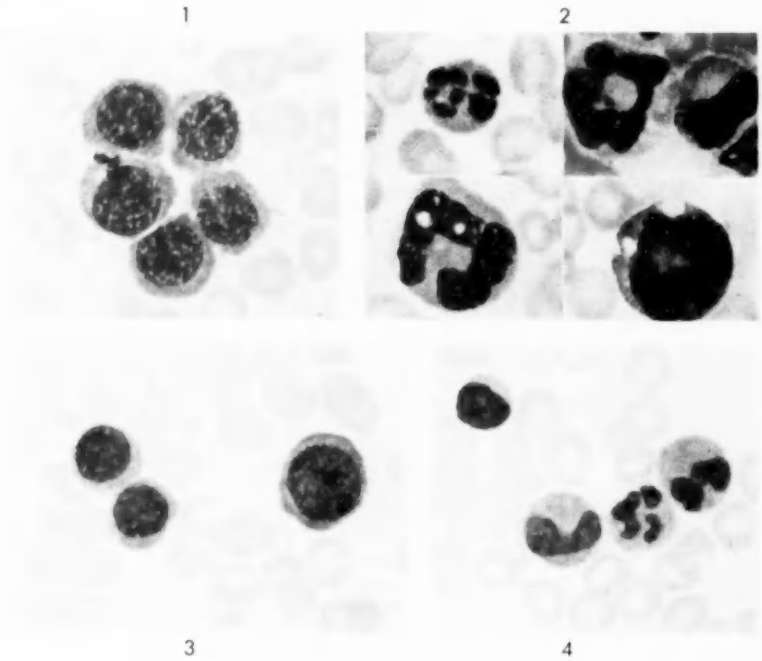

Plate I

FIG. 1. Sternal marrow from volunteer No. 4 taken 3 days after the last dose of pyrimethamine. Note the large early rubricytes of the erythroid series showing coarse granulations in the nuclei.

FIG. 2. Sternal marrow from same volunteer taken 3 days after last dose of pyrimethamine showing the enlarged cells of the myeloid series. Note the grotesque nuclei in the younger cells and the hypersegmentation in older cells plus the vacuolization.

FIG. 3. Sternal marrow from same volunteer taken 50 days after the last dose of drug. Cells are normal. Rubricytes are shown on the left and a prorubricyte on the right.

FIG. 4. Sternal marrow from the same volunteer taken 50 days after the last dose of drug showing normal cells of the myeloid series.

STUDIES IN HUMAN MALARIA

XXXIV. Acquired Resistance to Pyrimethamine (Daraprim) by the Chesson Strain of *Plasmodium vivax*

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As pointed out in a review by Bishop (1951), drug resistance often occurs during the course of treatment of some parasitic infections. Among the antimalarials, chlorguanide induces a relatively rapid development of resistance in laboratory strains of malaria when used in repeated subcurative dosages (Bishop and Birkett, 1947; Williamson *et al.*, 1947; Hawking and Perry, 1948; Schmidt *et al.*, 1949; Hawking and Thurston, 1951; Jaswant Singh *et al.*, 1952). Resistance to chlorguanide was also demonstrated in experimental human malaria by Seaton and Adams (1949), Seaton and Lourie (1949), and Cooper *et al.* (1950).

It was recently reported that malaria parasites can also develop resistance to pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine), an antimalarial having properties somewhat similar to chlorguanide. Rollo (1952) demonstrated that *Plasmodium gallinaceum* and *P. berghei* became resistant to pyrimethamine when the infections were treated with subcurative dosages. Schmidt and Genther (1953) developed a strain of *P. cynomolgi* which was a thousandfold more resistant to pyrimethamine than the parent strain. The resistance persisted after mosquito passage.

Studies reported in this paper were designed to test for the development of pyrimethamine resistance by the Chesson strain of *P. vivax*.

MATERIALS AND METHODS

The subjects used in this study were healthy adult white male volunteers from the inmate population of the U. S. Penitentiary, Atlanta, Georgia, who had no previous history of malaria. Many of the procedures used have been reported in an earlier paper (Coatney *et al.*, 1948). The parent strain had not been subjected to either pyrimethamine or chlorguanide prior to the beginning of the resistance studies. Volunteers were infected by the intravenous inoculation of approximately 1,000,000 malaria parasites from patient donors. Heparin was used as the anticoagulant and 0.85 per cent saline as the diluent.

Treatment with oral doses of pyrimethamine was begun on the third day of patent parasitemia. Each subject was treated initially for seven days with a single daily dose of pyrimethamine known to be subcurative. This amount was doubled at the end of every seven days in a step-wise fashion as long as parasitemia persisted. Twenty-five milligrams daily for seven days was the highest dosage given. If, during a particular dosage regimen, the blood became parasite-free, the drug was discontinued until the reappearance of parasites at which time administration of the drug was resumed at the same dosage until a total of seven

daily doses had been given. When drug-resistant parasites were transferred to a new subject the initial treatment was generally at the same dosage employed at the time of transfer.

In testing for transfer of resistance by the sexual forms of the parasite, groups of 200 to 600 *Anopheles quadrimaculatus* mosquitoes were infected by allowing them to feed on a subject whose parasites were pyrimethamine-resistant. One lot of mosquitoes fed when the patient was receiving 12.8 mg. of pyrimethamine daily, a second lot when the dosage was 25 mg. daily, and a third lot was allowed to feed 4 days after the seventh 25-mg. dosage. Following the usual extrinsic incubation period, each lot of infected mosquitoes was allowed to feed on two clean subjects. When parasitemia became patent in the recipients, pyrimethamine was given at the same dosage employed at the time mosquitoes were infected.

In order to determine cross resistance between pyrimethamine and chlorguanide, several subjects, whose parasites were resistant to the 25-mg. dosage, were subsequently given 12.5 mg. chlorguanide daily for seven days, the amount known to be curative in nonimmune subjects infected via blood inoculation with the same strain of *P. vivax* (Copper *et al.*, 1950). The dosage of chlorguanide was increased to 100 mg. daily for seven days when the infection was not cured by the 12.5-mg. dosage.

In order to evaluate the significance of the resistance studies, it was necessary to determine the minimum effective and minimum curative doses of the drug against trophozoite-induced infections. Accordingly, groups of volunteers were given single daily doses of pyrimethamine for seven consecutive days; the dosage regimens began at 0.25 mg. and increased step-wise to 16 mg.

Daily examinations of the patients' blood for parasites were made by the conventional thick film technique, using Giemsa-stained preparations.

All subjects were observed closely for signs and symptoms of drug intoxication. In addition to daily clinical observations, frequent complete blood counts and urine analyses were done as long as any drug was given. The blood studies also included weekly hematocrit, hemoglobin, and methemoglobin determinations.

TESTS AND RESULTS

A. *Development of resistance to pyrimethamine.* Patients Nos. AP-307 and AP-309 (Figures 1 and 2) received a step-wise increase in drug dosage every seven days, beginning at 0.1 mg. and 0.2 mg., respectively, until the dosage level of 25 mg. was reached. Parasitemia still persisted in subject AP-309 after receiving 25 mg. daily for seven days. Subject AP-307 was cured by the fifth 25-mg. dose.

That the pyrimethamine-resistant trophozoites can be transferred to other subjects is well illustrated by subject AP-319 who received parasitized blood from AP-309 (Figure 2). Nine days later when the parasitemia became patent, AP-319 was given 12.8 mg. of pyrimethamine daily for a total of seven days. This dosage resulted in a decrease in parasitemia but was not curative. After 6 daily doses of 25 mg. the blood became parasite-free and upon recrudescence 13 days later, the seventh dose was given. Parasitemia rapidly increased

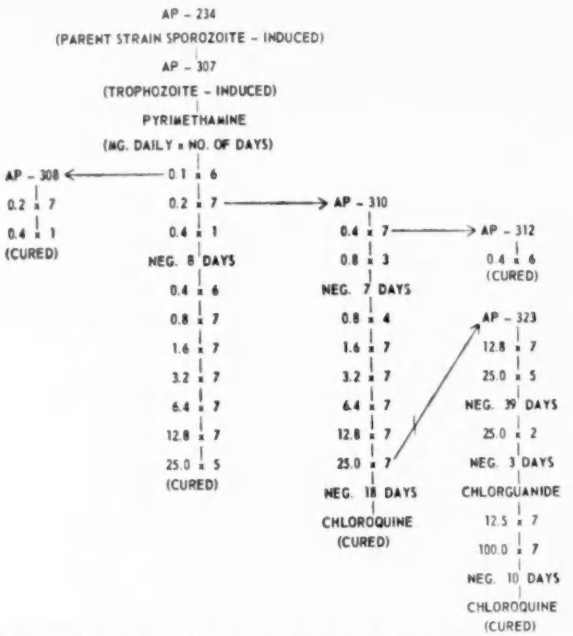

FIG. 1. Diagram showing the development of resistance to pyrimethamine in subjects AP-307, AP-308, AP-310, AP-312, and AP-323.

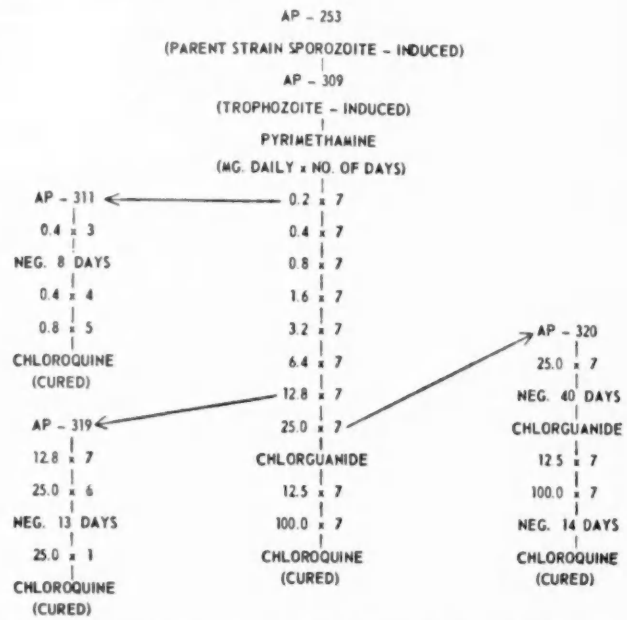

FIG. 2. Diagram showing the development of resistance to pyrimethamine in subjects AP-309, AP-311, AP-319, and AP-320.

and the infection was subsequently terminated with chloroquine (1.5 gm. base in 3 days). Similarly, subjects AP-310 and AP-323 (Figure 1) and AP-320 (Figure 2) were not cured by seven doses of 25 mg. of pyrimethamine.

B. *Transfer of pyrimethamine resistance through the mosquito.* The results of this study are shown in Figure 3. Subjects AP-321 and AP-322 were inoculated by the bites of mosquitoes previously infected by feeding on AP-309 who was harboring parasites resistant to 12.8 mg. of pyrimethamine. Subjects AP-324, AP-325, AP-332, and AP-333 were infected by using mosquitoes fed on the same subject when the malaria parasites were 25-mg. resistant. All 6 subjects developed patent parasitemia 12 to 16 days after the mosquito bites.

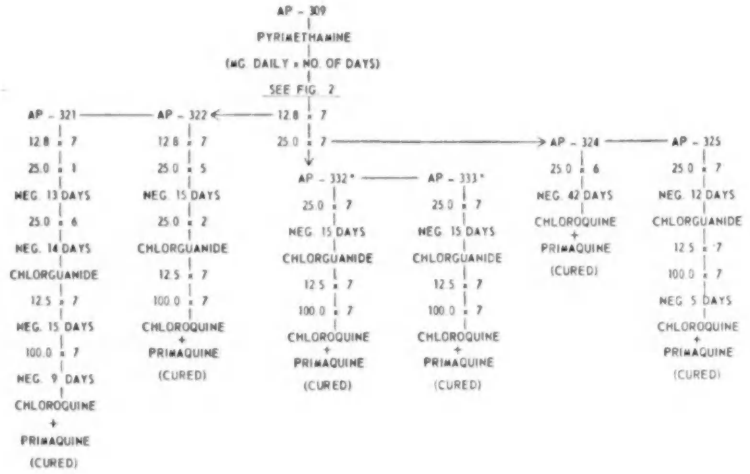

FIG. 3. Outline showing the transmission of pyrimethamine resistance through the mosquito.

Parasitemia persisted in AP-321 and AP-322 after they had received 12.8 mg. pyrimethamine daily for seven days and the dosage was increased to 25 mg. All 6 subjects were cleared of parasites by the 25 mg. daily dosage, but after stopping the drug, parasites reappeared (Figure 3).

C. *Response of the pyrimethamine-resistant strain to chlorguanide and to chloroquine.* As reported by Cooper *et al.* (1950), the minimum curative dose of chlorguanide against blood-induced Chesson strain *vivax* malaria is 12.5 mg. daily for seven days and 1.5 mg. produced detectable effect in the parasitemia. Consequently, the infections in AP-309, AP-320, and AP-323, refractory to 25 mg. of pyrimethamine daily for seven days, were treated with 12.5 mg. of chlorguanide daily for seven days in an effort to check for cross resistance. Parasitemia persisted in each of these subjects during this regimen and thereupon the dosage

was increased to 100 mg. of chlorguanide daily for seven days. At this dosage the parasitemia disappeared in AP-320 and AP-323, and was appreciably reduced in AP-309 (Figures 1 and 2). When the drug was stopped the parasites reappeared in the former subjects and increased in the latter.

Five sporozoite-induced infections resistant to 25-mg. dosage of pyrimethamine (AP-321, AP-322, AP-325, AP-332, and AP-333) were also treated initially with 12.5 mg. of chlorguanide daily for seven days. In 4 of the 5 subjects the parasitemia increased during drug administration while in the other, AP-321, parasites were absent from the circulating blood for a period of approximately two weeks after the last dose of drug. When the dosage of chlorguanide was increased to 100 mg. daily for seven days, the parasitemia persisted in AP-322, AP-332, and AP-333, and was temporarily eliminated in AP-321 and AP-325 (Figure 3).

TABLE 1

Effect of increasing dosages of pyrimethamine against blood-induced Chesson strain vivax *malaria*

NUMBER OF VOLUNTEERS	DAILY DOSE (MG.) FOR 7 DAYS	DAYS TO CLEAR PARASITEMIA	RESULTS
8	0.25	6,9,11,11,9,10,7; one not cleared	None cured; 7 recrudesced in 13,29,13, 9,9,9,13 days
8	0.5	9,7,11,11,10,7,6,10	2 cured; 6 recrudesced in 10,11,12,11, 13,13 days
8	1.0	4,8,7,7,10,8,8,5	5 cured; 3 recrudesced in 11,9,17 days
8	2.0	8,8,6,6,2,1,6,8	6 cured; 2 recrudesced in 14,15 days
4	4.0	10,2,8,6	2 cured; 2 recrudesced in 11,9 days
4	8.0	2,3,6,6	All cured
4	16.0	7,6,5,2	All cured

All infections not cured by pyrimethamine or by chlorguanide were finally terminated with chloroquine (1.5 gm. base) or chloroquine (1.5 gm. base) plus primaquine (15 mg. base daily × 14). The response to chloroquine was as rapid as in nonresistant infections; parasitemia was cleared by the third day in all cases.

D. *Minimum effective and minimum curative doses of pyrimethamine.* In evaluating the effect of pyrimethamine on blood-inoculated malaria it was necessary to determine the minimum effective dose (MED) (that amount of drug which, when given in a single dose daily for seven days, will reduce or temporarily clear the parasitemia below the perceptible level) and the minimum curative dose (MCD) (that amount of drug which, when given in a single dose daily for seven days, will cure the infection).

Forty-four subjects were inoculated with trophozoites of the parent strain and divided into seven groups, eight subjects in each of the four lower dosage groups and four subjects in each of the higher dosage groups (Table 1). The lowest dose tried, 0.25 mg., temporarily cleared the parasitemia in 7 of 8 patients treated and was therefore considered the minimum effective dose (MED). The infection was

cured (eradicated) in some patients at each dosage level from 0.5 up to and including 4.0 mg., but cure of the infection in all treated subjects was obtained only at the two highest dosages; 8.0 mg. was considered the minimum curative dose (MCD).

E. *Laboratory studies.* No toxic reactions were noted in any of the subjects given pyrimethamine. The slight decreases in hemoglobin and hematocrit as well as the changes in the white cell counts were those usually encountered in cases of malaria. Methemoglobin levels and urine analyses remained normal in all subjects.

DISCUSSION

The present studies indicate that the Chesson strain of *P. vivax* develops resistance to pyrimethamine when the parasites are exposed to subcurative doses over prolonged periods of time. The rate of development of resistance by the plasmodium can be determined by examining Figures 1 and 2. The fact that small doses of pyrimethamine cured the infections in subjects AP-308 and AP-312 (Figure 1) is significant. AP-308 was cured after receiving the first dose of 0.4 mg. (total of 1.8 mg.) probably because the donor parasites from AP-307 had not been exposed to the lower dosage long enough for the parasites to become resistant. The same is probably true of subject AP-312; even though the parasites had been subjected to pyrimethamine for more than two weeks, 0.4 mg. daily for six days (2.4 mg.) cured the infection. In the case of AP-307 (Figure 1) the infection was cured by 25 mg. of pyrimethamine daily for five days despite the fact that the parasites had been exposed to the drug for over two months.

It is obvious that the development of resistance by the parasite is slow and gradual and depends on carefully controlled increases in the amount of drug administered. If a critical amount is exceeded, as in patients AP-308 and AP-312, the parasites are destroyed before they develop further resistance. As indicated by the responses of subjects AP-310 and AP-323 (Figure 1) and AP-309, AP-319, and AP-320 (Figure 2), it would have been possible to go beyond the maximum dosage used, but with the definite demonstration of a more than 25-fold increase in resistance, it was not considered necessary to carry our studies further.

That pyrimethamine resistance was transferred through the mosquito is amply demonstrated in six subjects, but when the results of treatment with 25 mg. dosages are considered, an interesting development presents itself. The 25 mg. daily dosage resulted in the clearance of parasitemia for 12 to 42 days. The same result was obtained with five of the six blood-inoculated infections in whom the parasites built up to 25-mg. resistance; in one of the five, the infection was actually cured. Even though a many-fold resistance had been attained by carefully graded dosage increases, most of the parasites were still sensitive to the drug, and it appears that resistance will come slowly in the field if adequate dosages are employed.

The studies reported here as well as Schmidt's observations (1953) on *P. cynomolgi* indicate that pyrimethamine-resistant strains may show cross re-

sistance to chlorguanide. The fact that Jaswant Singh *et al.* (1952) demonstrated that chlorguanide-resistant strains of *P. knowlesi* were also resistant to pyrimethamine, and that Robertson *et al.* (1952) have shown the same type of cross resistance in *P. falciparum*, may add to the undesirability of using pyrimethamine for the treatment of acute malarial infections. If a strain were known to be resistant to either of these drugs, the best practice would be to employ chloroquine or other similar drugs.

In view of the flat dosage response curve exhibited by this drug, it might be argued that our selection of 8.0 mg. as the MCD was based on insufficient evidence because (1) some cures were obtained at all dosages down to 0.25 mg. and (2) although all infections were cured at 8.0 and 16.0 mg., failures might have occurred if we had employed more patients at these higher dosages. There is no denying this latter possibility, but it was felt that the problem did not warrant the use of additional volunteers.

SUMMARY AND CONCLUSIONS

A more than twenty-five-fold resistance to pyrimethamine was induced in the Chesson strain of *Plasmodium vivax* by treating blood-inoculated infections with gradually increasing dosages of the drug over prolonged periods. This acquired resistance was transferable by the mosquito. These pyrimethamine-resistant infections were also resistant to chlorguanide but remained as sensitive to chloroquine as the parent strain. Because the development of resistance was obtained only after careful planning and execution, it appears doubtful if resistance will become a serious problem under field conditions.

Acknowledgments

We wish to extend special thanks to Mr. James V. Bennett, Director of the Federal Bureau of Prisons; to Mr. William H. Hiatt, Warden of the U. S. Penitentiary, Atlanta, Georgia; to Dr. H. M. Janney, Chief Medical Officer of the prison; and to their respective staff members who made these studies possible.

We also wish to acknowledge an indebtedness to the Medical Service of the Army for providing some of the laboratory and nursing personnel for the project and to Dr. George Hitchings of the Wellcome Research Laboratories, Tuckahoe, New York, for supplying the pyrimethamine for use in these studies.

It would be difficult to overemphasize the contribution made by the inmate volunteers who willingly took the drug. We extend our sincere thanks to them for their contributions to this study.

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TREATMENT OF MALARIA WITH ORAL OR INTRAVENOUS PLAQUENIL

A Preliminary Report[1]

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On the north coast of Honduras, malaria is endemic all year, although of slightly decreased incidence during the dry season from May to September. Because of this endemicity, the adult native population may be considered as a relatively immune group. Previous studies in this area (Hoekenga, 1952) have indicated that almost every instance of *Plasmodium falciparum* or *Plasmodium vivax* malaria can be satisfactorily treated with a single oral dose of amodiaquin or chloroquine; only nine treatment failures were noted among 600 individuals so treated. Good as this record is, the search must go on for more efficient antiplasmodial drugs. This paper is a preliminary report on our work with another 4-aminoquinoline compound.

Plaquenil is 7-chloro-4-[4-(N-ethyl-N-β-hydroxyethylamino)-1-methylbutylamino] quinoline diphosphate. It has also been referred to as WIN 1258. Its synthesis was reported by Surrey and Hammer in 1950. The pharmacologic properties are similar to those of other 4-aminoquinoline drugs (Dennis *et al.*, in press). Acute intravenous, intraperitoneal and oral toxicity studies in mice show that Plaquenil is only about one-half as toxic as chloroquine diphosphate by the intravenous and intraperitoneal routes and is only one-fifth as toxic when administered orally. Studies of the blood levels following oral administration to dogs showed that Plaquenil is absorbed much more rapidly and gives blood levels several times higher than chloroquine diphosphate; significant concentrations are present in the plasma within one hour and persist for at least six hours. Blood level studies following the intramuscular administration of Plaquenil to dogs showed that the drug is rapidly absorbed and that significant concentrations are present in the plasma within 15 minutes. As is also true with chloroquine and amodiaquin, there is an unequal distribution of Plaquenil in the body tissues, the concentration in some tissues being much higher than in blood plasma. These several experimental studies crystallize the fact Plaquenil is at least as effective as chloroquine in treatment of malaria and is far less toxic.

Loughlin *et al.* (1952) were favorably impressed by their observations on 75 *P. falciparum* infections treated with Plaquenil in Haiti. The drug was given in single oral doses of 2 gm. to adults, 1.5 gm. to children six to twelve years of age, and 1.0 gm. to children under six years of age. Garzo Brito (1952) and his associates in Mexico City treated 91 individuals from several parts of Mexico with six dosage schedules; their conclusions are optimistic, but the number of patients on each dosage schedule is too small to draw definite conclusions. Clark (1953), in

[1] The Plaquenil used in this study was supplied by Winthrop-Stearns, Inc.

Panama, has been using a weekly suppressive treatment with tablets containing 30 mg. primaquine and 300 mg. Plaquenil; his preliminary results are also encouraging. These few preliminary clinical studies indicate that Plaquenil shares the following characteristics with chloroquine and amodiaquin: (1) acute attacks of malaria due to either *P. falciparum*, *P. vivax* or *P. malariae* are quickly terminated; (2) *P. falciparum* infections are usually completely eradicated; (3) *P. vivax* relapses are decreased in number and frequency; (4) there is very little effect on gametocytes.

MATERIAL AND METHODS

This study is based on the treatment of 82 persons who reside in or near the Tela Railroad Company (United Fruit Company) headquarters at La Lima, in northwestern Honduras. All patients were hospitalized. The ages ranged from seven years to sixty-four years. Sixty-two were males and twenty were females.

TABLE 1

*Immediate results of treatment of malaria with a single dose of Plaquenil**

	NO. OF CASES	AVERAGE HOURS FOR ATTAINMENT OF NORMAL TEMPERATURE	AVERAGE HOURS FOR DISAPPEARANCE OF PARASITEMIA
Single oral dose of 1.25 gm. Plaquenil			
P. falciparum	19*	20.4	30.6
P. vivax	47	18.1	23.0
Single intravenous dose of 0.36 Gm. Plaquenil			
P. falciparum	14	22.3	24.0

* Two immediate failures of *P. falciparum* patients treated with the oral dose of Plaquenil are not tabulated here.

All were of mestizo stock, and represented various mixtures of Indian, Negro and Spanish ancestry.

A single oral dose of 1.25 gm. was given to 21 patients with *P. falciparum* infection and to 47 patients with *P. vivax*. An additional fourteen individuals with *P. falciparum* were treated with 0.36 gm. intravenously; in all of these instances, the drug was included in 500 cc. of normal saline solution.

The route of administration (in *falciparum* infections) and the time of administration were not related to the parasite density, to the stage of the parasite cycle observed in the blood film or to the degree of fever. The oral temperature was recorded every four hours on all patients. Thick blood films were examined every six hours.

RESULTS

Immediate results. With the oral and the intravenous treatment regimens there was usually rapid clinical improvement. In the average case, sustained normal temperature was attained well within 24 hours and the blood was cleared of

parasites within 31 hours. There were two *P. falciparum* patients who did not respond to the oral dose at all, and who had to be re-treated. These results are given in Table 1.

Late results. A 20 year old girl with *P. falciparum* who had responded very favorably to the intravenous dosage was readmitted with clinical and parasitologic relapse after a three weeks' interval. She was re-treated with the identical dosage and has not relapsed in a 6 months' follow-up. This is the only known relapse among the 82 patients.

Toxicity. There were no apparent effects on the hemoglobin, red blood cell count or white blood cell count in any of the cases. Albuminuria or other suggestive evidence of renal damage was not observed in any instance. On ten patients receiving the oral medication and on ten patients receiving the intravenous dose, serum bilirubin, bromsulphalein clearance and Hanger cephalin-cholesterol flocculation were determined before treatment, the day after treatment and one week after treatment; in no instance was there evidence of liver function impairment. In none of the 14 *P. falciparum* cases receiving the intravenous medication was there appreciable change in the pulse or in the arterial blood pressure. There were no instances of dizziness, drowsiness, nausea, vomiting or visual disturbance. No adverse effects were noted on patients in pregnancy, with severe malnutrition or severe anemia.

SUMMARY

Eighty-two Hondurans were treated for malaria with 7-chloro-4-[4-(N-ethyl-N-β-hydroxyethylamino)-1-methylbutylamino] quinoline diphosphate (Plaquenil). Sixty-eight of them (21 with *P. falciparum*, 47 with *P. vivax*) were given a single oral dose of 1.25 gm. Fourteen others with *P. falciparum* infections were given an intravenous dose of 0.36 gm. in 500 cc. of normal saline solution. Both the immediate and the late results were encouraging. Toxic side-effects of the drug were not encountered. Plaquenil, therefore, warrants further investigation; its experimental use will be continued in this and other locations.

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CHLOROQUINE AND CHLORGUANIDE AS SUPPRESSANTS OF MALARIA IN GUATEMALA[1]

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A cooperative project to evaluate the efficacy of certain antimalarial drugs under field conditions in Guatemala was begun in August 1948, under the auspices of the Pan American Sanitary Bureau and the Republic of Guatemala. When the project began, relatively little information was available on the subject of drug control of malaria, but the results of field experiments using new antimalarial drugs in different parts of the world appeared in the literature soon after (for review see Cooper, W. C., 1949; Coatney, G. R., 1951; Schmidt, L. H., 1952). However, owing to the differences in objective procedure, the geographical location of each experiment and the different strains of plasmodia dealt with, it seemed advisable to complete the investigations. This paper deals with the results of studies made from August 1948 to July 1950.

The Republic of Guatemala lies between 13° 44½′ and 17° 49½′ latitude and 88° 14′ and 92° 13′ west longitude, and is bounded on the west and north by Mexico, on the east by British Honduras, the Gulf of Honduras, Spanish Honduras and El Salvador, and on the southwest and south by the Pacific Ocean. Most of the area is mountainous, in some places reaching an altitude of over 13,000 feet. The area is estimated to be 45,452 square miles. The capital, Guatemala City, at an elevation of 5,000 feet, with a population of about 280,000, is the largest city. The country is divided into 22 states or *departamentos*.

TOPOGRAPHY AND GEOLOGY OF GUATEMALA AS RELATED TO MALARIA

Guatemala may be divided into six physical regions, which reflect the underlying geology of the country. The areas differ in climate and rainfall, with consequent differences in flora and fauna. These variations are noticeable in their effect on malaria prevalence, which is determined to a large extent by rainfall and temperature, which are again dependent on altitude and physical orientation. It should be noted that Guatemala is a rather small country, and that great differences in climate may occur within relatively short distances.

The Pacific coastal region (*costa*) extends inland 20 to 35 miles, and is largely an alluvial plain, traversed by numerous small rivers which arise in the highlands. The soil of this plain nearest the Pacific is black, heavy and silty, very wet and sticky during the rains, but cracked and fissured during the dry season. Along the coast and up the rivers are mangrove swamps and dense jungle growth. Farther inland, the plain is open pastures and cultivated fields, with occasional

[1] These investigations were conducted with the support of the Laboratory of Tropical Diseases, National Microbiological Institute, National Institutes of Health, under the sponsorship of the Pan American Sanitary Bureau, through a grant from the Division of Research Grants, National Institutes of Health.

small groves of large trees. The river banks are lined with dense undergrowth and high forest. The coastal plain gradually rises northeastward through a piedmont area. The plains suffer extremes of dry and wet seasons, and are moderately malarious in some parts.

The second region, composed of highlands formed by a gently curving arc of high young volcanic cones, extends from Volcan Tajumulco in the State of San Marcos to Volcan Tajual in Jutiapa, near the border of El Salvador. Severe erosion is very prevalent in the highlands owing to the high rainfall and the loose nature of the soil, abetted by the prevailing agricultural practices of the inhabitants. There is some malaria in the submontane region, which becomes progressively less at higher altitudes.

Inland from the younger volcanic highlands is the third region, formed by more ancient and eroded volcanic mountains, the backbone of the Continental Divide. The climate here is cool, the soil is fertile, and the rainfall abundant. Many level plateaus alternate with deeply eroded gorges and ravines. Owing to the high altitude and cool climate, malaria is prevalent only in some small isolated areas.

The fourth region is formed of interior valleys between mountain ranges running from west to east. The principal rivers, including the Cuilco, Negro and Motagua, flow nearly eastward to the Atlantic Ocean. Some of the valleys, cut off from moisture-laden winds from the oceans, are dry most of the year. Malaria varies in intensity with the rainfall, being lowest in the drier valleys.

The fifth region is the eastern coastal plain, extending inland from the irregular shoreline of the Gulf of Honduras. The lower valleys of the Rio Motagua and Rio Polochic are in this area. The climate is prevailingly tropical, with high rainfall. The area is somewhat malarious.

The sixth region includes the vast area of Petén, which is bounded on the southwest by the Altos Cuchumantanes, a great fault plain which rises over 3,000 feet above the basins of the Cuilco and Negro rivers. From this great limestone massif, and from the lower altitudes in the State of Alta Verapaz to the east, the terrain slopes gradually northward to the broad, flat plain of Petén, which is actually an extension of the plains of Campeche in southern Mexico. The rainfall is light and the incidence of malaria is low. Those interested in a further account of the geology and geography of the country are referred to Sapper (1894, 1925, 1932), Schuchert (1935), and McBryde (1947).

CLIMATE AND METEOROLOGY

Three overlapping climatic regions are recognized: hot, 0 to 3,200 feet; temperate, 3,200 to 6,550 feet; and cold, over 6,550 feet. Two seasons, dry and wet, occur in most of the country. Exceptions may be found along the eastern coast and in the State of Alta Verapaz, where there is usually considerable precipitation during the entire year. In most of the country the wet season is from May to November; however, in the monsoon belt, between 2,300 and 4,500 feet along the Pacific slope, the rainy season is from March to December.

Few temperature and humidity records are available. In the Pacific lowlands the average relative humidity is about 70 per cent during the dry season and 85 per cent during the wet season. Seasonal fluctuations in temperature are slight,

but diurnal extremes are often marked. The average temperature on the coast at San José is 82°F., and at Escuintla, 1,000 feet above sea level and 30 miles inland, it is 78°F. In Guatemala City, at 5,000 feet, the average daily temperature ranges from 42° to 86°F., and averages 64°F.

The annual rainfall is as low as 20 inches in the more arid regions, to over 200 inches on parts of the Pacific slope (Table 1 and Figure 1). General rains are infrequent, much of the rain falling as local showers. Precipitation is usually heaviest in the months of June and September. On the Pacific slope, from sea level to 3,000 feet, a direct relation exists between precipitation and altitude. Table 1 shows this relation at a few selected points. North of the arc of the highest mountain chain the annual rainfall approaches that found in the Pacific lowlands. An example of the relation of rainfall to the incidence of malaria in a selected area during our survey period is shown in Figure 3.

TABLE 1

Relation of altitude to rainfall and temperature in southern Guatemala, based on records from 1930 to 1950

STATION	ALTITUDE ABOVE SEA LEVEL (FEET)	ANNUAL RAINFALL (INCHES)			AVERAGE ANNUAL TEMPERATURE (°F.)
		Low	High	Average	
San Jose	6	26.4	88	55	82
El Obero	99	19.5	75	48	81
La Maquina, Finca	244	68	125	89	—
Santa Maria	400	55	127	91	80
San Victor, Finca	594	81	141	91.5	—
Escuintla	1,112	42	155	111	78
Concepcion, Finca	1,600	87	207	136	76
Amatitlan	3,900	29.6	72	41	68
Guatemala City	5,000	29.6	72	49	64

POPULATION STATISTICS

According to the lastest official government report (1950 unpublished) the population of Guatemala was 2,787,000. About 60 to 70 per cent of the people are Indians, and most of the rest are "ladinos", mixed Indian and white, or Indians who are culturally Spanish (McBryde, 1947). Most of the Indians live in the highlands.

The State of Escuintla, where most of our studies were made, has a population of about 190,744, mostly *ladinos*. The urban population is 23,508 and the rural population 167,236. Most of the latter live on plantations or ranches (both called *fincas*), in houses grouped around the home of the owner or manager. The largest city is Escuintla, the capital of the State, with a population of about 7,000; the second largest town, San José, has about 4,000 people.

ECONOMIC STATISTICS

About 80 per cent of the population is engaged in agriculture. The most important export crops are coffee, bananas, citronella and sugar.

Judged by United States standards the average annual income is very low, poverty is widespread and illiteracy is high. In addition to their wages the laborers on the *fincas* are provided with quarters for their families and a small plot of land for corn and garden crops. The extra help required to harvest seasonal crops is provided by itinerant Indians and *ladinos* who work part of the year on

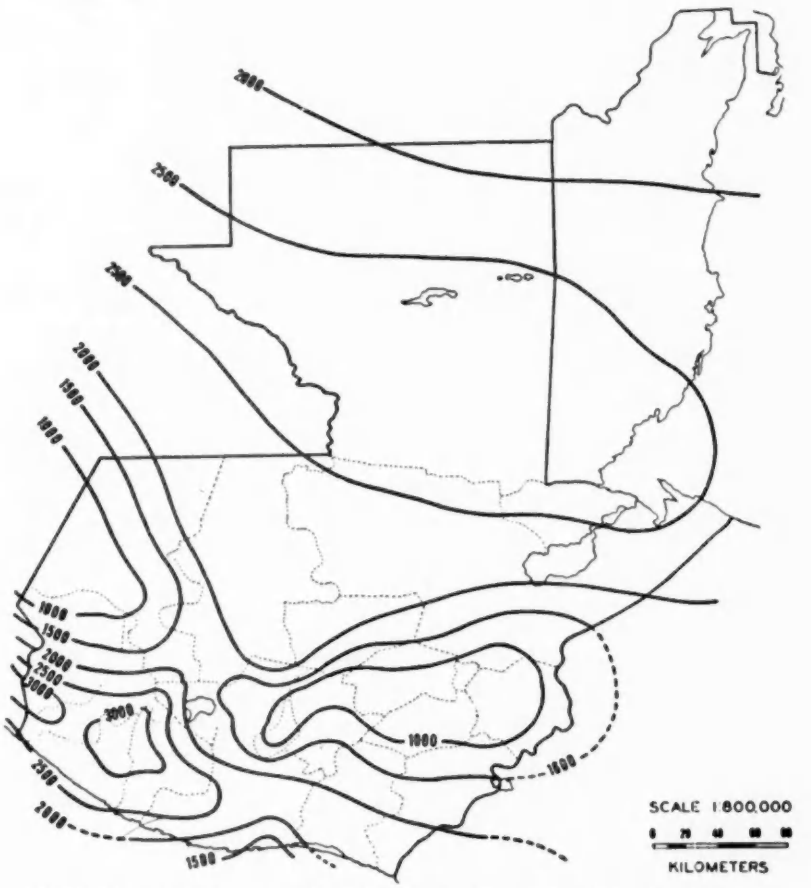

FIG. 1. Map of the Republic of Guatemala showing annual rainfall in millimeters

the *fincas*, and then return to their homes in the highlands. Because of cheap and rapid autobus transportation, the rise of new industries in the cities, the construction of new highways and the seasonal nature of employment on the farms, the population is fluid. This condition made it difficult for us to find a stable group for our studies.

VITAL STATISTICS

The average annual death rate (Kolbe, 1948) for the years 1943 to 1947 is estimated at 19.6 per 1,000; birth rates for the same period are over 40 per 1,000. Shattuck (1938) estimated a mortality rate of 19.9 per 1,000. No true figure for morbidity or mortality can be established because illness is rarely treated by a physician; consequently, the cause of death as recorded in the "Registro Civil" is often incorrect. In the hospitals the leading causes of death are tuberculosis, respiratory diseases, dysenteries (enteritis) and malaria. All recent surveys indicate that, except for a few isolated instances, endemic malaria in Guatemala is confined to the lowlands, where most of the deaths are attributed to malaria.

HISTORY OF MALARIA IN GUATEMALA

As in all Central America, malaria seems to have been a serious problem since the Spanish conquest. Shattuck (1938) states that a mild type may have been indigenous. The annual reports of the United Fruit Company (1919–1931) show an incidence as high as 47 per cent in the Quiriguá region near the Gulf of Honduras. The first systematic malaria survey was made from 1928 to 1933 under the direction of Mira (1936). In his investigations, limited to children, he reported spleen indices up to 100 per cent, but the parasite index, for most places, was about the same as reported in this paper. *Plasmodium vivax* was listed as the most prevalent species, but there were some foci of *P. falciparum* and *P. malariae*. Herrera (1940) and co-workers of the Public Health Service of Guatemala (*Sanidad Publica*) continued the work initiated by Mira through 1939, extending the surveys to other parts of Guatemala, making some 11,472 blood examinations. The overall parasite incidence (largely in school children) was 28.4 per cent at altitudes from 0 to 820 feet, 23.4 per cent from 820 to 3,280 feet, and 4.0 per cent over 3,280 feet. The time of year when the surveys were made was not indicated. The ratio of the frequency of the three species of plasmodia was two *P. vivax* to one *P. falciparum* to one *P. malariae*. In the highlands *P. vivax* was the usual species found. The Public Health Service of Guatemala continued to make malaria surveys from 1940 to 1949, but the results have not been published. Spleen and parasite indices found during this period were made available to us. These records indicate that there has been a general reduction in the incidence of malaria all over Guatemala, including the areas in which no control measures have been undertaken. The newer antimalarial drugs are generally available, and since 1949 extensive mosquito abatement projects have been in operation in certain malarious areas.

Anopheles albimanus and *A. pseudopunctipennis* are the most common species of mosquito reported from the malarious areas by both Mira (1936) and Herrera (1940). *A. albimanus* has the widest distribution and was most prevalent. It has been reported in Guatemala City at an elevation of 5,000 feet.

A. pseudopunctipennis was reported at 4,800 feet by De Leon (1936) and it was believed to be the carrier of malaria in Santa Catarina on the shore of Lake Atitlan, (over 6,000 feet) where autochthonous cases were found in 1930 (Shat-

tuck, 1938). This species was found at the lake town of Panajachel by Komp, 1950 (personal communication).

A. darlingi is found in northeast Guatemala in the Polochi-Lago Izabal region and Brennan (1951) reports having found *A. crucians* near the town of Amatitlán.

METHODS AND PROCEDURES

The survey team usually consisted of three or four people. The first person examined the spleens, the second recorded the name of each examinee, including the results of his spleen examination, age, sex, recent history of chills and fever or other illness, and the number of the blood slide; the third person made the blood smears, and the fourth, needed when large groups were examined, kept the people assembled, entertained the children and otherwise assisted in the work.

Spleen examinations were usually made once a month on a scheduled drug administration day. These were done by one person (W. C. W.) after the method of Hackett (1944). Spleen examinations were not made during the 1948 surveys because no one trained in making these examinations was available. Thick blood smears were made and stained with Giemsa after the method of Barber and and Komp (1924), usually on the day after they were taken. All the blood films were examined in Guatemala by technicians of the United States Public Health Service. Usually 100 fields were examined before a slide was considered negative. The presence of gametocytes, as well as other stages of the parasite, was recorded.

Attendance at the first and second surveys was usually high, but subsequent attendance dropped sharply in spite of considerable persuasion. Even when there was full cooperation of the owners of plantations and officials of the villages, it was necessary to employ various devices to sustain interest among the people. Lotteries and raffles were held with some success. At times the winning prizes were given to those with the largest spleens or the highest parasite count. During the period of drug administration, prizes were given for attendance. Where electricity was available, moving pictures were shown from time to time to stimulate interest and increase the attendance rate. In spite of all these methods, however, it finally became necessary to carry on the work "house to house."

Drug administration. Two blood surveys were made during the month preceding the start of the drug-testing program. During the period of treatment blood surveys were made every two weeks for the first two months after the program started, and thereafter once a month. One week after the last treatment was given, a final blood survey was made. Two or more blood examinations were made during the follow-up period of about three months after treatment was stopped.

Every effort was made to make sure that test individuals actually took the drug. The adults and older children swallowed the tablets with water; small children and infants were given crushed tablets in water on a spoon. All treatments were given or supervised by members of the team. Candies were given to the children to mask the bitter taste of the drugs. Drugs were given on a

scheduled day of each week. Control groups were given placebos that resembled the drug.

Before an individual was given the drug, the roster was checked for a recorded prior smear. In the event none was recorded, a smear was made and then the drug was administered. Each drug administration was recorded on a field sheet after the name of each person. Reasons for refusal to take the drug, failure to retain it, as occurred in some infants, and reactions to the drug were also recorded.

OBSERVATIONS

Malaria parasites were found in 3,106 or 13.27 per cent of the 23,390 blood films taken during the surveys (Tables 2 and 3); splenomegaly was found in 1,474 or 19.41 per cent of the 7,694 examinations made (Table 4). The indices given in the tables are not a true index of malaria in Guatemala because most of the surveys were made in the malarious regions of the lowlands; examinations were few in the generally malaria-free highlands. Probably a more correct picture of the overall incidence of malaria may be obtained from a perusal of Tables 2, 3, 4 and Figure 2.

The monthly spleen and parasite indices in the State of Escuintla, where statistically significant numbers of people were examined in every month except February, 1949, shows the seasonal fluctuations of malaria in an endemic area (Figure 3 and Table 5). The spleen index was consistently higher than the parasite index, and a rise in parasitemia appeared to be followed by a rise in splenomegaly. The effect of rainfall on the rate of parasitemia was not observed over a sufficiently long period to warrant a positive statement; however, it can be said that the lowest parasitemia occurred in May just after the beginning of the rainy season and that two peaks of parasitemia occurred; the first, about six weeks after the start of the rainy season, the second, about six weeks after the rainy season ended (Figure 3).

Anopheles albimanus appeared to be the principal vector of malaria in the areas included in these studies. *A. pseudopunctipennis* apparently was not an important vector, as it is known to be in some of the Mexican highlands. The latter was not found within five miles of the Pacific coast, where *A. albimanus* was always present. A detailed report of the mosquito vectors will appear in a separate paper.

People of all ages were included in the surveys, for one purpose of the test was to determine the effect of treating entire populations. On the plantations the adults (over 15 years) outnumbered the children two to one. The parasite index was lowest in infants under one year and highest in children from 2 to 14 years of age. The spleen indices were highest in children, especially in the 10- to 14-year group, and lowest in the 1- to 2-year group.

The relative numbers of males and females examined varied somewhat from place to place. In villages and towns the sex ratio was about equal. On the plantations the populations were made up of approximately 45 per cent men, 20 per cent women, and 35 per cent children. About half of the laborers on the plantations were migrant workers whose families remained behind in the highlands or

TABLE 2

Monthly parasite indices of areas in the State of Escuintla, Guatemala, from 0 to 900 feet above sea level

MONTHS OF SURVEY	AREA I 0 TO 50 FEET				AREA II 50 TO 350 FEET				AREA III 350 TO 600 FEET				AREA IV 600 TO 900 FEET			
	Number		Per cent positive	Standard error	Number		Per cent positive	Standard error	Number		Per cent positive	Standard error	Number		Per cent positive	Standard error
	Examined	Positive			Examined	Positive			Examined	Positive			Examined	Positive		
1948																
Sept.	376	109	28.9	2.34	804	114	14.1	1.23	49	2	4.0	2.83	—	—	—	—
Oct.	—	—	—	—	651	120	18.4	1.52	688	42	7.9	1.60	—	—	—	—
Nov.	122	25	20.5	3.65	20	9	45.0	11.12	153	21	13.7	2.76	—	—	—	—
Dec.	449	92	20.4	1.91	112	19	16.9	3.55	26	2	7.6	5.23	312	27	8.6	2.44
1949																
Jan.	139	32	23.0	3.57	376	64	17.0	1.94	202	69	34.1	3.33	280	58	20.7	2.42
Feb.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mar.	49	13	26.5	6.30	222	45	20.2	2.70	—	—	—	—	—	—	—	—
Apr.	105	14	13.3	3.13	440	37	8.4	1.32	199	60	30.1	3.25	97	15	15.4	3.66
May	17	0	—	—	328	33	10.0	1.66	36	5	13.8	5.77	251	20	7.9	1.70
June	—	—	—	—	472	77	16.3	1.70	125	36	28.8	4.05	208	20	9.8	2.04
July	143	43	30.1	3.84	225	52	23.1	2.81	5	1	20.0	17.89	13	3	23.1	11.69
Aug.	472	109	23.0	1.94	543	72	13.2	1.46	75	10	13.3	3.92	343	45	13.1	1.82
Sept.	88	25	28.4	4.81	227	27	11.8	2.15	85	18	21.1	4.43	180	33	18.3	2.88
Oct.	68	8	11.7	3.91	—	—	—	—	—	—	—	—	206	36	17.4	2.65
Nov.	57	5	8.7	3.75	301	91	30.2	2.65	152	47	30.9	3.75	153	16	10.4	2.48
Dec.	112	27	24.1	4.07	792	149	18.8	1.39	180	31	17.2	2.81	221	27	12.2	2.20
1950																
Jan.	227	31	13.6	2.28	527	70	13.2	1.48	425	76	17.8	1.86	294	38	12.9	1.96
Feb.	93	10	10.7	3.22	485	70	14.4	1.59	444	100	22.5	1.97	185	17	9.1	2.12
Mar.	85	10	11.7	3.50	138	17	12.3	2.73	218	44	20.2	2.72	86	6	6.9	2.87
Apr.	66	8	12.1	4.01	129	13	10.1	2.65	118	35	29.6	4.21	53	5	9.4	4.01
May	71	4	5.6	2.73	63	12	19.0	4.94	9	2	22.2	13.85	137	4	2.9	2.92
June	52	10	19.2	5.46	84	16	19.0	4.28	68	29	42.6	6.00	33	0	—	—
Total examinations	2791	575	20.6		6939	1107	15.9		3257	630	19.3		3052	370	12.1	

TABLE 3

Parasite indices in areas of Guatemala where drugs were not administered

DATE	PARASITE INDEX			SPLEEN INDEX		
	Number palpated	Number positive	Per cent positive	Number palpated	Number positive	Per cent positive
Area V, Escuintla, altitude 900 to 3500 feet						
1948						
Sept.	32	5	15.6	—	—	—
Oct.	248	20	8.0	—	—	—
Nov.	1,311	37	2.8	—	—	—
Dec.	484	8	1.6	—	—	—
1949						
May	153	9	5.8	81	8	9.8
July	167	3	1.8	156	1	0.6
Aug.	16	2	12.5	16	0	—
Sept.	135	5	3.7	128	9	7.0
Nov.	47	9	19.1	—	—	—
Dec.	316	13	4.1	—	—	—
1950						
Jan.	157	2	1.2	117	8	6.8
Total	3,066	113	3.6	498	26	5.2
Area VI, Lake Amatitlan, altitude 3800 to 4200 feet						
1949						
Mar.	149	8	5.3	86	3	3.4
Apr.	401	38	9.4	365	33	9.0
June	113	27	23.6	92	30	32.6
July	124	15	12.0	11	0	—
Aug.	125	8	6.4	35	0	—
Sept.	78	5	6.4	34	11	32.3
Dec.	161	13	8.0	—	—	—
Total	1,151	114	9.9	623	77	12.3
Area VII, Santa Rosa (State), altitude 3800 to 4300 feet						
1949						
Jan.	128	3	2.3	—	—	—
Feb.	203	1	0.4	—	—	—
Apr.	239	5	2.0	224	4	1.7
Total	570	9	1.5	224	4	1.7
Area VIII, Lake Atitlan, altitude 5000 to 5500 feet						
1949						
May	186	0	—	184	2	1.0
Area IX, Southwestern Guatemala (coastal plain)						
1950						
Jan.	910	50	5.4	832	71	8.5

TABLE 3—*Continued*

DATE	PARASITE INDEX			SPLEEN INDEX		
	Number palpated	Number positive	Per cent positive	Number palpated	Number positive	Per cent positive
Area X, Alta Verapaz, altitude 1000 to 4300 feet						
1950 Mar.	655	69	10.5	665	123	18.4
Area XI, Jalapa-Chiquimula, altitude 2700 to 3400 feet						
1949 Jan.	303	29	8.9	—	—	—
Area XII, Peten, altitude 600 feet						
1949 Jan.	91	4	4.3	—	—	—
Area XIII, Izabal, altitude 8 feet						
1949 Jan.	110	0	—	—	—	—
Area XIV, Huehuetenango—Quiche, altitude over 4000 feet						
1948 Sept.	129	18	13.9	—	—	—
Guatemala City, Military Hospital						
1949 1950	63	12	19.0	—	—	—
San Jose, U. S. Air Force Troops and Natives, altitude 15 feet						
1948 Aug.	97	9	9.2	—	—	—
Total	7,331	427	5.8	3,026	303	10.0

towns. The malaria rate in males and females varied somewhat from place to place; in the lowlands it was lowest in the adult males and highest in women and children as is usual in tropical America. Spleen indices were also significantly higher in women than in men. The difference may be due to the free medical attention, including antimalarial drugs, which were more readily available to the workers than to their families.

The species incidence of all the identified parasites, exclusive of those found in individuals receiving drugs, were: *P. vivax* 51.5 per cent, *P. falciparum* 45.9 per cent, and *P. malariae* 2.6 per cent (Table 6). Mixed infections amounted to two per cent and undetermined species 16.8 per cent of the total parasites found. Mira (1936) and Herrera (1940), who confined their studies to children, found more *P. vivax* and *P. malariae*, but considerably fewer *P. falciparum* infections

than we. Along the Pacific coastal plain below 900 feet the species found in the 2,682 postives, including mixed infections, were: *P. vivax* 50.8 per cent, *P. falciparum* 46.8 per cent, and *P. malariae* 2.3 per cent. In the regions above 900 feet the distribution of species was: *P. vivax* 56.0 per cent, *P. falciparum* 39.5 per cent, and *P. malariae* 4.3 per cent.

P. vivax, which accounted for 51.5 per cent of the identified infections, was the predominant species in all areas except in Area I (Table 6) along the Pacific coast. On the Pacific coastal plain (Figure 4) *P. vivax* infections outnumbered the other species for 13 out of 21 months of the study period.

P. falciparum, found in 45.9 per cent of the identified infections, was the predominant species on the Pacific coast at altitudes from 0 to 50 feet above sea

TABLE 4

Results of spleen examinations in all areas

AREA	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE	SPLEEN SIZE				
				1	2	3	4	5
I	913	325	35.5	134	114	71	5	1
II	1,980	516	26.0	267	162	76	11	—
III	899	218	24.2	108	72	37	1	—
IV	876	112	12.7	64	37	11	—	—
Total	4,668	1,171	25.08	573	385	195	17	1
V	498	26	5.2	14	10	2	—	—
VI	623	77	12.3	40	24	9	1	3
VII	224	4	1.7	3	1	—	—	—
VIII	184	2	1.8	2	—	—	—	—
IX	832	71	8.5	44	16	6	5	—
X	665	123	18.4	27	46	46	4	—
Total	3,026	303	10.0	130	97	63	10	3
Grand total	7,694	1,474	19.41	703	482	258	27	4

level. Figure 4 shows that *P. falciparum* was the predominant species below 900 feet in 8 out of 22 months of the study.

P. malariae appears to have decreased from 25 per cent found by Mira in 1931 (Mira, 1936) to 2.6 per cent as reported here.

Mixed infections were found only 62 times, 2 per cent of the total examined, and only one slide showed a triple infection. *P. vivax* and *P. falciparum* were the species found in all but a few of the double infections.

THE SUPPRESSION EXPERIMENTS

The region below 900 feet in the State of Escuintla was chosen as the most suitable area for the drug evaluation tests because it had the highest incidence of malaria in a large and accessible population (Figure 5). Six sites (Figure 6),

each made up of three or more plantations (in one instance a small village adjacent to a plantation) were selected for the field trials. At each of the six sites part of the population was treated with chloroquine, part with chlorguanide and

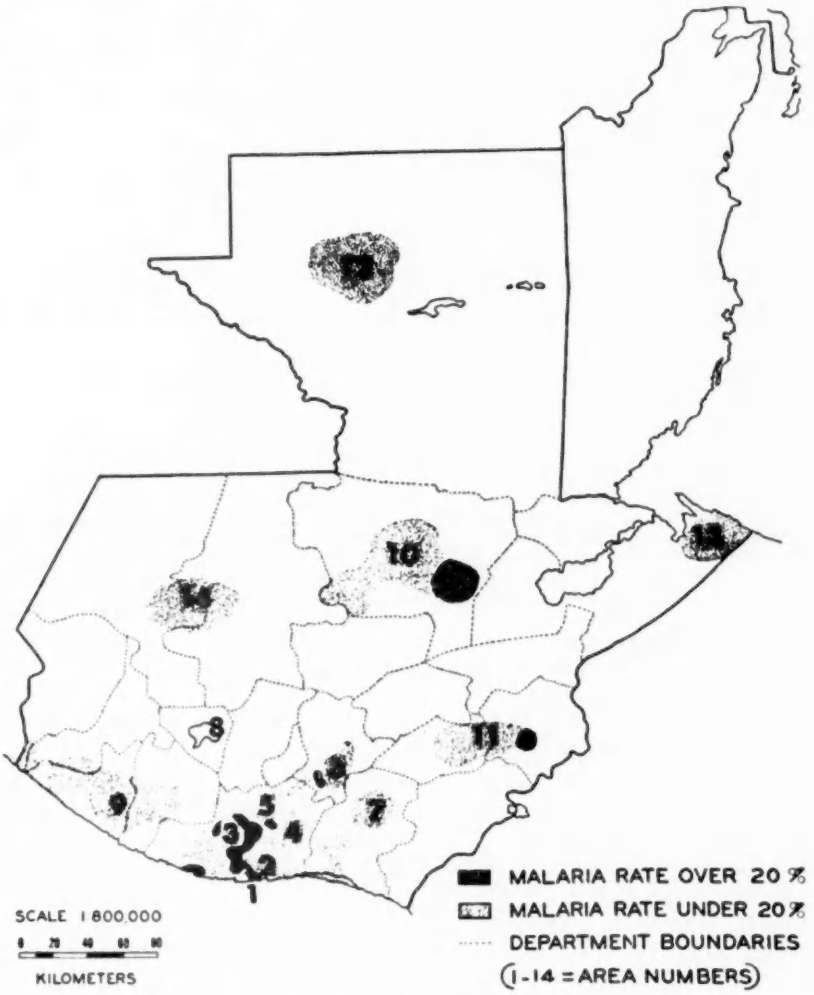

FIG. 2. Incidence of malaria in surveyed areas of Guatemala

the remainder served as the control. Each site was divided into areas and only one drug was administered at any given place. The topography, climate, population and malaria incidence of the units within each site were essentially iden-

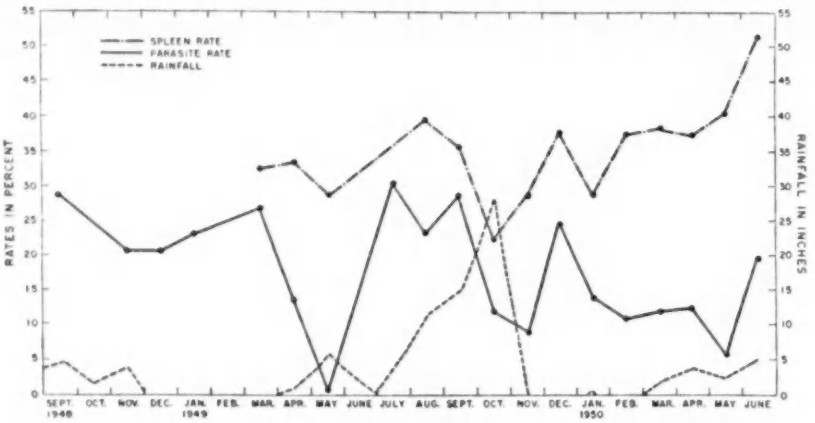

FIG. 3. The relation of rainfall to the incidence of malaria in the State of Escuintla

TABLE 5

Monthly spleen indices of areas in the State of Escuintla, Guatemala, from 0 to 900 feet above sea level

MONTHS OF SURVEY	AREA I 0 TO 50 FEET			AREA II 50 TO 350 FEET			AREA III 350 TO 600 FEET			AREA IV 600 TO 900 FEET		
	Number		Per cent positive	Number		Per cent positive	Number		Per cent positive	Number		Per cent positive
	Palpated	Positive		Palpated	Positive		Palpated	Positive		Palpated	Positive	
1949												
Mar.	49	16	32.6	222	74	33.3	—	—	—	—	—	—
Apr.	105	35	33.3	316	72	22.7	195	48	24.6	95	15	15.7
May	14	4	28.5	334	95	28.4	37	10	27.0	149	23	15.4
June	2	2	—	224	46	20.5	108	26	24.0	168	16	9.5
July	5	3	—	33	8	24.5	—	—	—	—	—	—
Aug.	104	37	35.5	72	12	16.6	29	1	3.4	—	—	—
Sept.	179	64	35.7	—	—	—	—	—	—	62	15	24.1
Oct.	36	8	22.2	—	—	—	—	—	—	—	—	—
Nov.	63	18	28.5	99	24	24.2	—	—	—	33	3	9.0
Dec.	58	22	37.9	22	6	27.2	—	—	—	40	4	10.0
1950												
Jan.	45	13	28.8	149	26	17.4	160	19	11.8	46	4	8.6
Feb.	51	19	37.2	160	69	43.1	159	41	25.7	131	13	9.9
Mar.	55	21	38.1	111	30	27.0	35	4	11.4	45	5	11.1
Apr.	59	22	37.2	90	23	25.5	78	30	38.4	44	4	9.0
May	47	19	40.4	78	17	21.7	32	13	40.6	30	3	10.0
June	41	22	53.6	70	14	20.0	66	26	39.3	33	7	21.2
Total examinations	913	325	35.5	1980	516	26.0	899	218	24.2	876	112	12.7

tical. The houses at a given site were within a radius of two and one half miles or less, and within the flight range of the same mosquito population. Approximately equal numbers of people were to have received chloroquine and chlorguanide; unfortunately, however, migrations caused some disproportionate totals (Table 7).

TABLE 6

Species and parasite indices found in 14 areas in Guatemala

AREA	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE	PLASMODIUM VIVAX	PLASMODIUM FALCIPARUM	PLASMODIUM MALARIAE	SPECIES UNDETERMINED	MIXED INFECTION
I	2,791	575	20.6	215	253	16	103	12
II	6,939	1,107	15.9	487	420	33	187	20
III	3,257	630	19.3	280	265	2	98	15
IV	3,052	370	12.1	180	132	3	61	6
Total	16,039	2,682	16.7	1,162	1,070	54	449	53
Per cent				50.8	46.8	2.3	16.8	1.9
V	3,066	113	3.6	53	39	4	18	1
VI	1,151	114	9.9	64	29	1	22	2
VII	570	9	1.5	5	2		2	
VIII	186	0						
IX	910	50	5.4	20	24	1	7	2
X	655	69	10.5	33	20	4	12	
XI	303	29	8.9	6	17		6	
XII	91	4	4.3	2		1	1	
XIII	110	0						
XIV	129	18	13.9	11	6	4	1	4
Total	7,171	406	5.6	194	137	15	69	9
Per cent				56.0	39.5	4.3	16.9	2.2
Grand total	23,210*	3,088	13.3	1,356	1,207	69	518	62
Per cent				51.5	45.9	2.6	16.8	2.0

* The examinations done at the U. S. Air Base, San José and the Military Hospital, Guatemala City are not included.

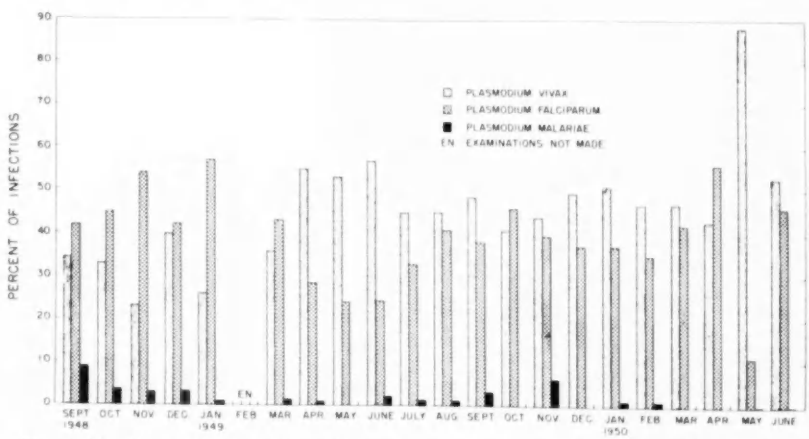

FIG. 4. Monthly incidence of malaria species in areas I, II, III, and IV below 900 feet

Groups 1, 2, and 3 received weekly drug treatments for 30 weeks; group 4 for 15 weeks; and groups 5 and 6 were treated once every two weeks for 16 weeks

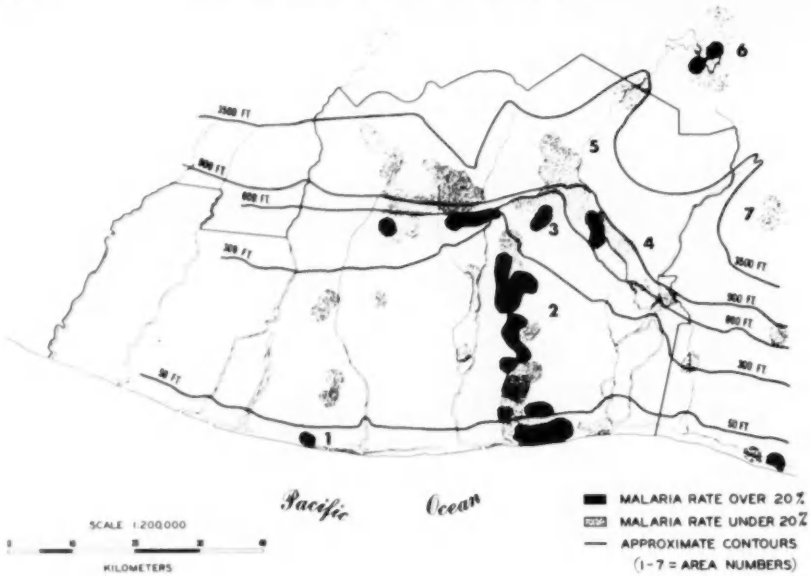

FIG. 5. Incidence of malaria in the State of Escuintla

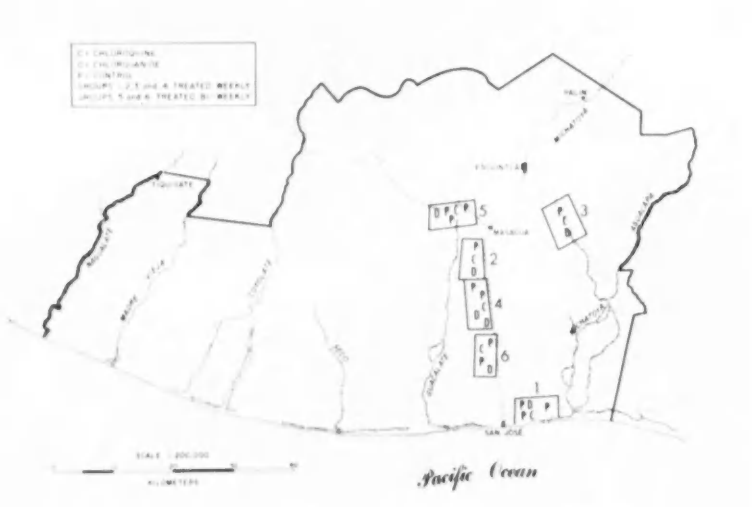

FIG. 6. Location of test groups in the State of Escuintla

(Figure 6). Prior to treatment 3,211 people (Table 7) were examined and placed on the rolls; this number dwindled to 2,087 by the end of the experiment. Those on the weekly regimens of chloroquine, chlorguanide, and placebo totaled 538,

TABLE 7

Division of the treated population into experimental groups

GROUP	TOTAL EXAMINED	EXPERIMENTAL GROUP			
		Total	Chloroquine	Chlorguanide	Control
Treated weekly					
1	942	695	365	186	144
2	212	110	33	60	17
3	520	354	113	132	109
4	602	399	27	305	67
Total	2,276	1,558	538	683	337
Treated biweekly					
5	676	388	176	49	163
6	259	141	53	24	64
Total	935	529	229	73	227
Weekly and biweekly groups					
Grand total	3,211	2,087	767	756	564

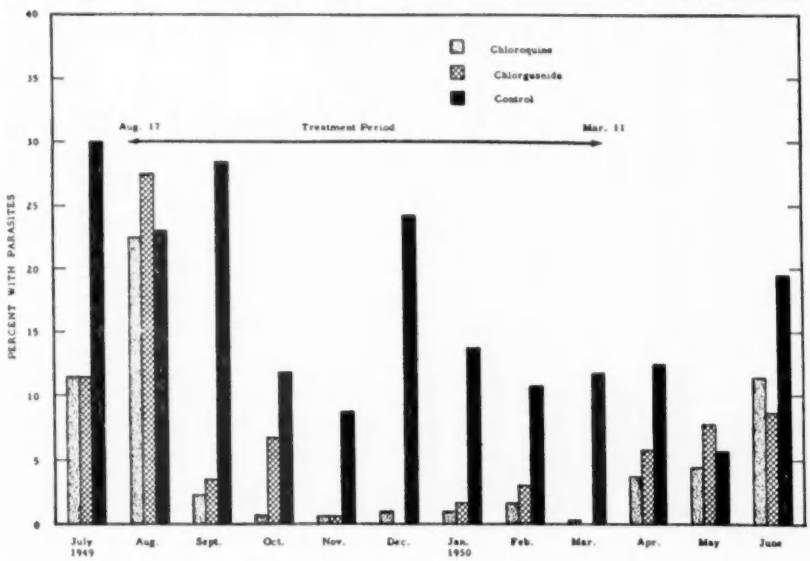

FIG. 7. Parasite indices in group 1

683, and 337, respectively. During the test period surveys were carried out at adjoining plantations. The combined results of these later surveys and of the control groups (Table 2 and Figure 3) give a good picture of the amount of uncontrolled malaria before, during, and after the administration of the drugs.

It was arbitrarily decided not to institute medication at any place until two conditions had been met: (1) a parasite rate of at least 20 per cent and (2) observation of a given location through one rainy and one dry season. As a consequence, experimental groups were started on treatments at different times (Figures 7 to 14). Owing to some differences in altitude, rainfall and the season of transmission in each area, the data from each experimental group are treated separately.

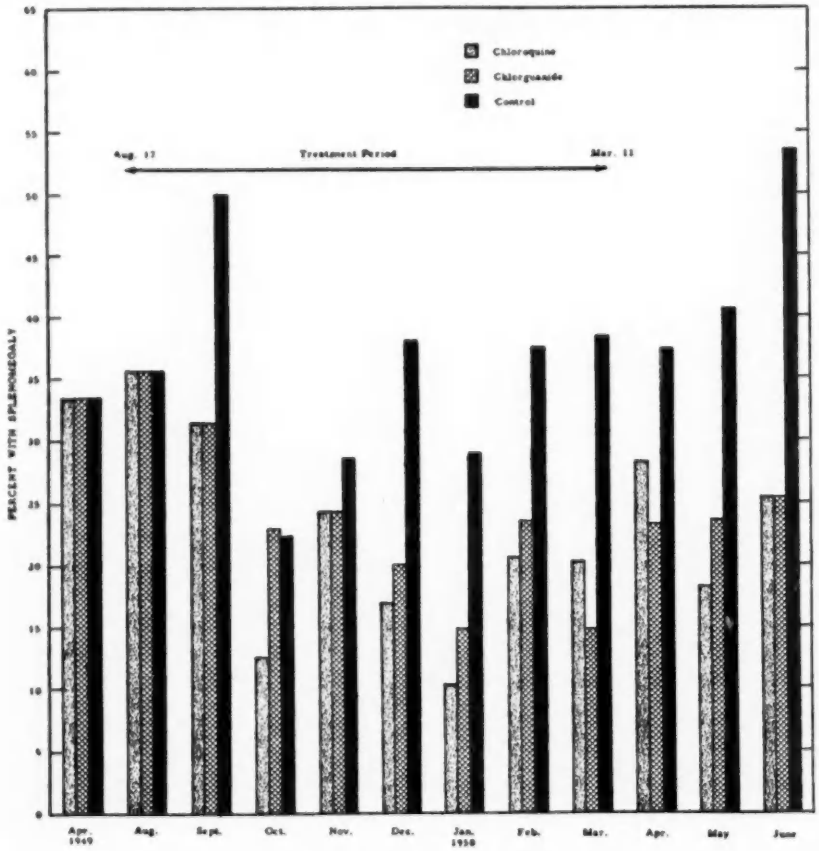

FIG. 8. Spleen indices in group 1

However, the results of the experiment were sufficiently comparable to warrant combining the data from all areas for the purpose of drawing general conclusions.

SUPPRESSIVE REGIMENS

Weekly treatments.

A. Chloroquine diphosphate, 300 mg. (base) single dose.
B. Chlorguanide monohydrochloride, 300 mg. (salt) single dose.
C. Placebo.

Biweekly treatments.

A. Chloroquine diphosphate, 300 mg. (base) single dose.
B. Chlorguanide monohydrochloride, 300 mg. (salt) single dose.
C. Placebo.

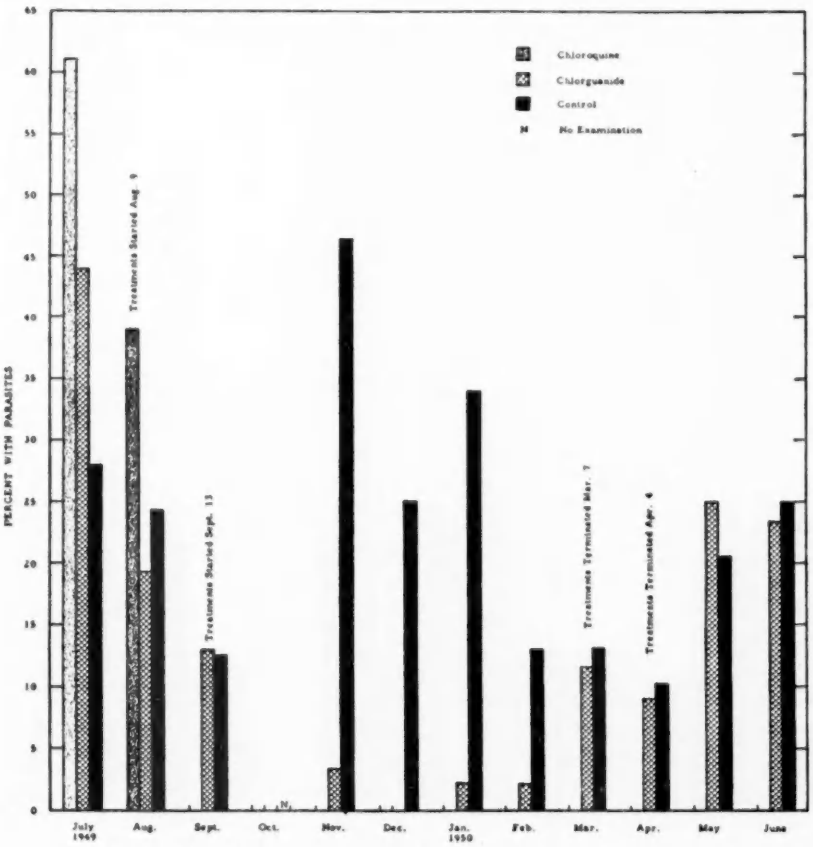

FIG. 9. Effect of chloroquine and chlorguanide treatments in group 2

Dosages by age.

12 years and over—full dose, 300 mg.
6 to 11 years—½ dose, 150 mg.
2 to 5 years—¼ dose, 75 mg.
less than 2 years—proportional to weight.

When overt cases of malaria appeared in individuals under Regimen A or B they were treated with the same drugs as given for suppression. Overt cases among the controls were treated with 15 grains of quinine daily for three days. At each

location an attempt was made to treat the entire population. Temporary employees, visitors and migrants were often treated, especially if they requested it, but in no instance were they included in the experimental group. During the

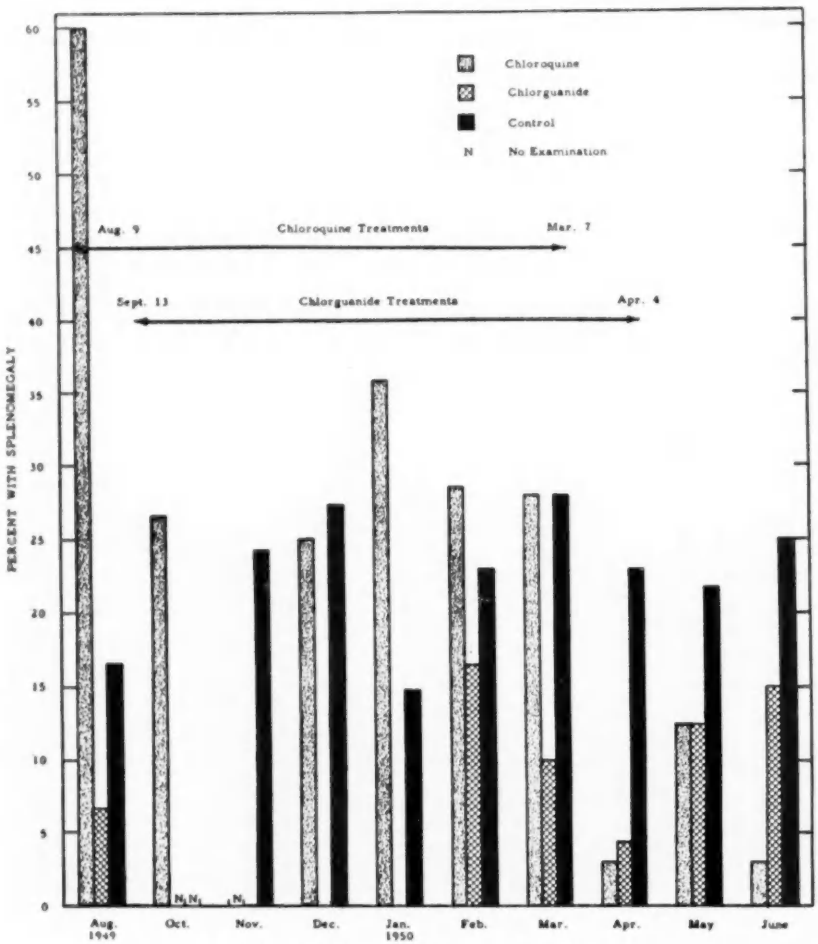

FIG. 10. Spleen indices in group 2

treatment period all new employees were examined for malaria and were treated thereafter.

Due to heavy rains and floods (*temporal*) in October 1949, the main highways became impassable and many people were forced to vacate their homes temporarily. During this period one or more treatments were missed; other irregularities were for the most part the result of the migratory habits of the people.

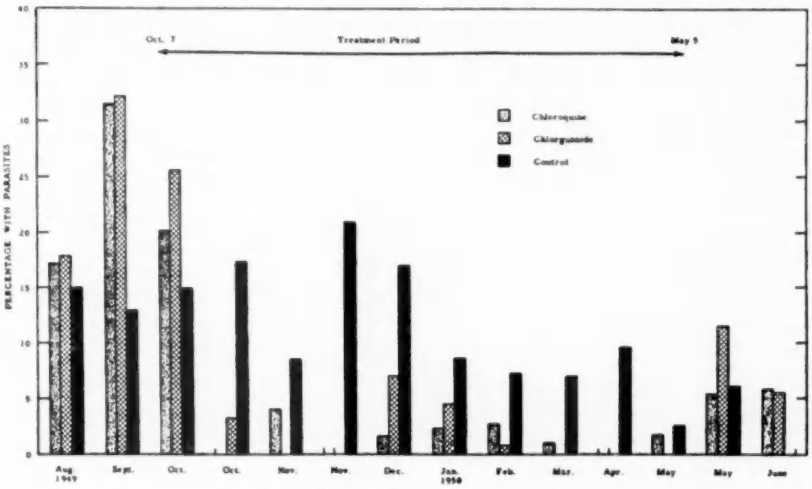

FIG. 11. Parasite indices in group 3

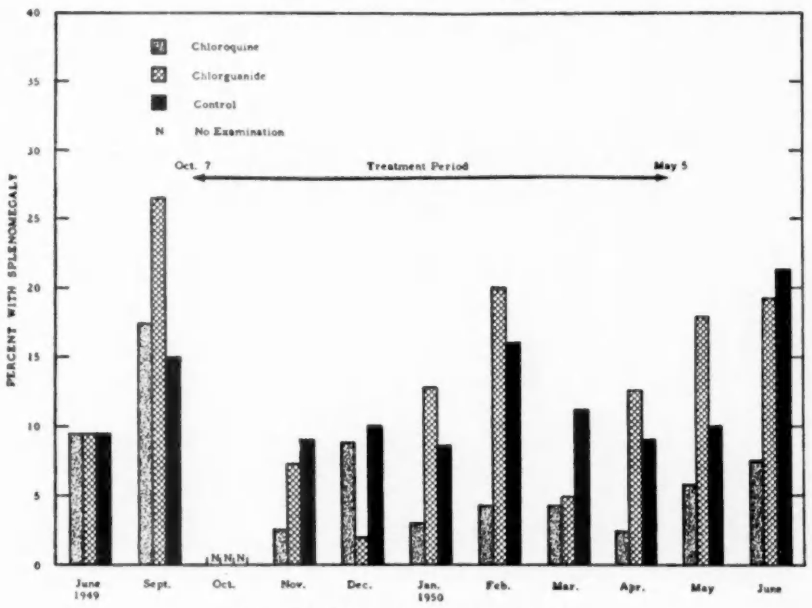

FIG. 12. Spleen indices in group 3

At one time, 25 people on the chlorguanide regimen in group 3 (Figures 11 and 12, and Table 10) received one dose of chloroquine by error. Data on this group were compared with those of "regular" test groups; no statistically significant difference was noted.

A blood smear was made from each person on the day he received his first suppressive medication and only in rare instances were people given their first treatment without having been recently examined for malaria parasites. Such individuals were not included in the base-line for comparative analyses of malaria incidence prior to medication. About 70 per cent of each test group received treatment as scheduled.

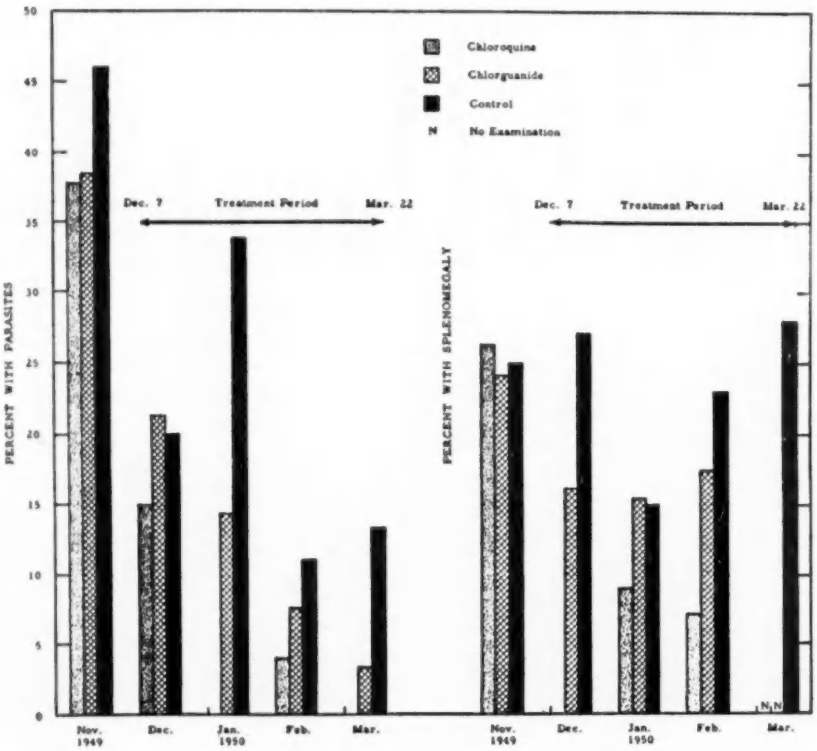

FIG. 13. Parasite and spleen indices in group 4

RESULTS OF THE EXPERIMENTS

Treatment of the "entire population" with chloroquine and chlorquanide according to the above regimens resulted in marked reduction but not complete suppression of clinical malaria and parasitemia. In some areas there appeared to be a reduction in the spleen indices and in the size of the average enlarged spleen during the treatment period.

Monthly blood and spleen indices in each experimental population taken before, during and after treatment are shown in Figures 7 to 14. In each figure dates of the first and last treatments are indicated by arrows. The results of the

surveys prior to the first treatment are shown in the first group of three columns. The second set of columns shows the parasite indices on the day first treatments were given. The third set of columns shows the indices one month after treatment started, and so on. On the weekly programs, blood parasite surveys were made one week after the final drug administration; on the biweekly programs, corresponding surveys were made two weeks after the last medication was given. Each figure also shows the trend of malaria following discontinuance of suppressive treatment.

The results of the treatments, based on the actual number of individuals found positive[2] at each site, are summarized in Tables 8 to 12. The pretreatment period,

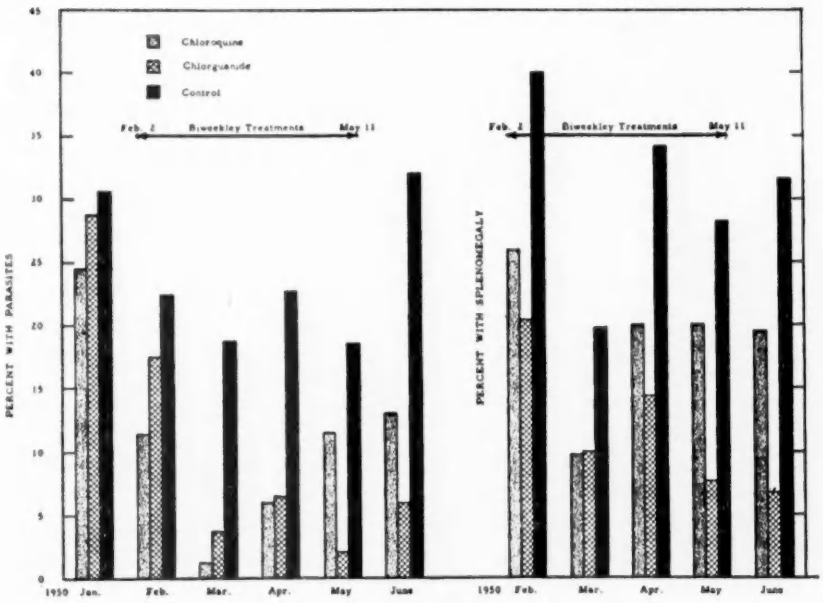

FIG. 14. Parasite and spleen indices in groups 5 and 6

comparable to a base-line, includes the results of the blood examinations made just prior to, or on the day the first treatment was given. A spleen survey was also made at or about the same time. The results shown in Tables 8 to 12 were compiled from the monthly (or more frequent) surveys made during the treatment period. Some people were admitted to the program after the first dose of drug had been given, but these are not included in the data unless they were on treatment long enough to demonstrate the effectiveness of the drug. The post-treatment period shows the percentages of parasite-positive individuals found in

[2] If the same individual was positive more than once (and one was positive five times) he was counted only *once*.

TABLE 8

Effects of chloroquine and chlorguanide on malaria parasite indices and spleens in Group 1

REGIMEN	PRE-TREATMENT	TREATMENT	POST-TREATMENT	INDIVIDUALS ON EACH REGIMEN
Chloroquine				365
Parasite index (per cent)	22.7	4.3	11.8	
Spleen index (per cent)	35.3	24.6	31.3	
Average enlarged spleens	0.45	0.37	0.43	
Chlorguanide				186
Parasite index (per cent)	23.0	9.1	12.5	
Spleen index (per cent)	35.3	29.9	28.4	
Average enlarged spleens	0.51	0.45	0.46	
Placebo				144
Parasite index (per cent)	23.0	35.4	21.1	
Spleen index (per cent)	35.3	39.8	48.6	
Average enlarged spleens	0.61	0.80	0.88	

TABLE 9

Effect of treatment on malaria parasite indices and spleens in Group 2

REGIMEN	PRE-TREATMENT	TREATMENT	POST-TREATMENT	INDIVIDUALS ON EACH REGIMEN
Chloroquine				33
Parasite index (per cent)	38.7	0.0	0.0	
Spleen index (per cent)	60.0	32.0	36.3	
Average enlarged spleens	0.95	0.60	0.72	
Chlorguanide				60
Parasite index (per cent)	18.3	13.3	36.3	
Spleen index (per cent)	10.7	11.3	10.7	
Average enlarged spleens	0.10	0.11	0.10	
Placebo				84
Parasite index (per cent)	23	29.7	22.8	
Spleen index (per cent)	11.1	30.3	32.3	
Average enlarged spleens	0.11	0.57	0.52	

TABLE 10

Effect of treatment on malaria parasite indices and spleens in Group 3

REGIMEN	PRE-TREATMENT	TREATMENT	POST-TREATMENT	INDIVIDUALS ON EACH REGIMEN
Chloroquine				113
Parasite index (per cent)	22.3	7.9	3.5	
Spleen index (per cent)	12.5	7.1	11.6	
Average enlarged spleens	0.18	0.09	0.13	
Chlorguanide				132
Parasite index (per cent)	21.6	9.0	11.7	
Spleen index (per cent)	25.0	20.7	16.6	
Average enlarged spleens	0.39	0.23	0.25	
Placebo				109
Parasite index (per cent)	12.6	18.3	5.2	
Spleen index (per cent)	11.6	21.8	19.3	
Average enlarged spleens	0.13	0.23	0.29	

the surveys made (in the follow-up period) after treatment was suspended. The letters AES refer to the average enlarged spleen of a population at a given period or examination (Hackett, 1944).

TABLE 11

Effect of treatment on malaria parasite indices and spleens in Group 4

REGIMEN	PRE-TREATMENT	TREATMENT	INDIVIDUALS ON EACH REGIMEN
Chloroquine			27
Parasite index (per cent)	33.3	7.4	
Spleen index (per cent)	28.5	12.5	
Average enlarged spleens	0.35	0.12	
Chlorguanide			305
Parasite index (per cent)	26.1	24.2	
Spleen index (per cent)	17.6	21.2	
Average enlarged spleens	0.28	0.32	
Placebo			67
Parasite index (per cent)	29.1	48.8	
Spleen index (per cent)	11.1	30.3	
Average enlarged spleens	0.11	0.57	

Post-treatment surveys were not made in Group 4.

TABLE 12

Effect of treatment on malaria parasite indices and spleens in Groups 5 and 6, Areas III and II, respectively

REGIMEN	PRE-TREATMENT	TREATMENT	POST-TREATMENT	INDIVIDUALS ON EACH REGIMEN
Chloroquine				229
Parasite index (per cent)	18.0	13.1	16.6	
Spleen index (per cent)	30.5	23.2	10.8	
Average enlarged spleens	0.49	0.33	0.13	
Chlorguanide				73
Parasite index (per cent)	18.3	6.8	5.5	
Spleen index (per cent)	17.2	12.7	6.4	
Average enlarged spleens	0.25	0.14	0.06	
Placebo				227
Parasite index (per cent)	26.6	28.3	31.0	
Spleen index (per cent)	35.0	38.8	35.7	
Average enlarged spleens	0.61	0.64	0.70	

Group 1, Area I

Three hundred sixty-five people were treated with chloroquine, 186 with chlorguanide, and 144 served as controls. In the period from September, 1948, to August, 1949, 44 per cent of the people examined were positive for malaria one or more times. The last pretreatment survey for blood parasites was made in August (Figure 7). In the chloroquine-treated group (Table 8) the parasite index, including that of persons admitted to the program late, dropped from 22.7 per cent in August to 4.3 per cent during the treatment period, and then rose to 11.8 per cent in the post-treatment period.

The parasite index in the chlorguanide group dropped from 23 per cent in August to 9.1 per cent during the treatment period, and then rose to 12.5 per cent in the post-treatment period.

On each survey the parasite index of the untreated controls was consistently higher than that of the treated groups. The monthly indices of the control group followed the seasonal fluctuations for the Pacific coastal areas (Figure 3). The parasite index of the controls changed from 23 per cent in August to 35.4 per cent during the treatment period, to 21.1 per cent in the post-treatment period. This group (Group 1) was the only one in which *Plasmodium falciparum* was the predominant species in all but three of the monthly surveys from August, 1948, to June, 1950.

Splenomegaly was found in 342, or 42.2 per cent of 790 individuals examined in surveys made from March, 1949, to September, 1949.

Treatments in Group 1 did not markedly reduce the incidence of splenomegaly (Figure 8). In the dry season (April, 1949) the spleen index for this group was 33 per cent; in the rainy season, on the last pre-treatment survey in August, the index was over 35 per cent. The monthly indices in the post-treatment period show a slight rise in the spleen indices in persons treated with chloroquine. Throughout the treatment period the indices in the controls were uniformly higher than in the test groups. In both treated groups there was a slight fall in spleen index, while in the control group there was slight rise, indicating medication had some effect on the spleen rate.

In comparison with the pretreatment period (Table 8) there was a slight decrease in the AES in the chloroquine- and chlorguanide-treated groups. On the other hand, the AES of the control group increased, indicating that there was probably less active malaria in the groups under suppression.

Group 2, Area II

This group was so divided that only 33 were treated with chloroquine, 60 with chlorguanide, and 84 served as controls. Chloroquine treatments were started in August and chlorguanide treatments a month later (Figure 9). In this area the lowest parasite rate occurred in April; the rainy season is longer than along the coast. Forty per cent of the population examined in all the surveys prior to the first medication had positive bloods one or more times. Including those who came on the program late, the parasitemia of the chloroquine group (Table 9) was reduced from 38.7 per cent on a survey taken just before drug administration, to zero during the treatment and follow-up periods. In the chlorguanide group the parasite index was reduced from 18.3 per cent before treatment, to 13.3 per cent during the treatment period, and rose to 36.3 per cent in the post-treatment period. In March, April, May and June the parasite indices of the control and chlorguanide groups were almost equal (Figure 9). Because some of the control areas for Group 4 were located near Group 2, they were used as controls for both experimental groups.

The spleen index of the chloroquine group (Table 9) decreased from 60 per cent in the August survey to 32.0 per cent during treatment, and rose to 36.3 per cent

in the post-treatment period. The AES for the corresponding periods was reduced from 0.95 to 0.60, and then rose to 0.72. The spleen index in the chlorguanide group was about 11 per cent in all periods; no spleens larger than size 1 were found. In August before treatment started, 11.1 per cent of the controls (Groups 2 and 4) had splenomegaly; and AES was 0.11 per cent. During the treatment period the spleen index rose to 30.3 per cent, and the AES to 0.57 per cent. In the post-treatment period the AES and the spleen index showed no appreciable change from the preceding period.

Group 3, Area IV

This group was located between 600 and 900 feet, where the average blood parasite and spleen indices were low (Tables 2 and 3). However, the three plantations which formed Group 3 were located near swamplands, and some surveys showed parasite and spleen indices of about 40 per cent, which is comparatively high for Guatemala. The 354 people making up the group were divided into three nearly equal groups (Table 7, 10); 113 were given chloroquine, 132 were given chlorguanide, and 109 were controls. In the surveys of this group from January to September 1949, 33.8 per cent had positive bloods one or more times, and 23.6 per cent had palpable spleens one or more times.

The results of this experiment are shown in Table 10 and Figures 11 and 12. The monthly parasite index shows a decline in the treated groups. However, the parasite index in May and June, 1950, following treatment, was as high as the corresponding months in 1949, indicating that treatment had not affected the general malaria incidence in this group. Among both the chloroquine and chlorguanide groups there was a decrease in the parasite index during the treatment period and a slight increase in the control group during the same period. After treatment was terminated there was a further decrease in parasite index in the chloroquine group, a slight rise in the chlorguanide group and a distinct decrease in the controls, indicating that there probably was little transmission during this period.

During the treatment and post-treatment period there was a rise in spleen index and AES of the control group. In the chlorguanide group there was a slight decrease in the spleen index and AES in both treatment and post-treatment period. In the chloroquine group there was a more distinct decrease in spleen index and AES during treatment with a slight rise in both during the post-treatment period.

Group 4, Area II

Owing to irregular attendance, occasioned in part by the migration of the transient workers in March, 1949, this group was treated weekly for only 15 weeks. Of the 399 people in this group the individuals treated with chloroquine, chlorguanide, and placebos were 27, 305, and 67, respectively. In the surveys from October, 1948, to November, 1949, about 42 per cent of this group had malaria parasites one or more times, and in the pretreatment period 33.7 per cent of those examined showed splenomegaly one or more times. The monthly blood

surveys (Figure 13) indicate a very gradual and slow response to treatment with chlorguanide.

The percentages of individuals under each regimen who had malaria parasites and splenomegaly, for the periods just before and during treatment, appear in Table 11. Those on the chloroquine regimen showed a significant reduction in the parasite and spleen indices, and in the size of the AES. The control group showed an increase in the parasite and spleen indices and in the size of the AES. In those on the chlorguanide regimen, 74 or 24.2 per cent had parasitemia during the treatment period. Just before treatment was started there appeared to be an epidemic of malaria on the plantation, for the incidence rose from 10 per cent to 42 per cent in six weeks. The relative incidence of *P. falciparum* rose from 25 per cent to 68 per cent. Changes in the spleen index and in the AES among the chlorguanide group were not significant.

Groups 5 and 6, Areas III and II

Because of the small number of individuals in Group 5, Area III, and Group 6, Area II, it was decided to combine them in making this report. In these groups the drugs were administered every two weeks. Before medication was begun the average parasite and spleen indices and the relative proportions of each species of plasmodia were about the same in both groups, but during treatment the parasite index in the controls was much higher in Groups 5, Area III than in Group 6, Area II.

The monthly parasite and spleen indices (Figure 14) show little change in the control group during the entire period of observation. Biweekly medication with chloroquine appeared to give less satisfactory results than chlorguanide. In both drug regimens reduction in the spleen index was slight when compared with the indices found in the base-line survey made in February 1949.

Of the 529 people on the biweekly programs, 229 were given chloroquine, 73 chlorguanide and 227 placebos. Migrations and poor cooperation resulted in the unequal numbers placed on the different regimens. In the surveys made in the year preceding administration of the drugs, 40 per cent of the population had parasite-positive blood smears one or more times, and 39 per cent of those examined had enlarged spleens one or more times. The data on the individuals on each regimen, in the periods just before, during and after treatment, are summarized in Table 12.

The parasite and spleen indices as well as the AES (Table 12) on the controls showed very little change during the three periods. Under the chloroquine and chlorguanide regimens there were reductions in the spleen index and in the size of the AES. Chloroquine given biweekly did not appear to reduce the parasite index markedly. On the other hand, in the group receiving chlorguanide biweekly there was a more marked drop in the incidence of parasitemia. Unfortunately, the number on this regimen (Table 7) was too small to be of much statistical value. Also, it was discovered that on one plantation under this regimen some antimalarial measures, including medication with chloroquine and chlorguanide, had been carried out by the owner. It appeared that satisfactory suppression of

malarial parasites was not obtained by biweekly treatments with chloroquine. However, there appeared to be more clinical malaria in the groups receiving the placebo than in the treated groups.

Consolidated data from the groups on weekly regimens. In order to show the overall suppressive effect when chloroquine and chlorguanide are given weekly, the data from all groups were combined.

Parasite and spleen indices according to the number of treatments. In many studies of this nature all persons carried on the register are included in the analysis of results irrespective of the number of treatments actually taken; some may have taken the test drug only twice while others took all or nearly all the regimen required. For this analysis the individuals included in each regimen were arranged in groups according to the number of treatments received (Table 13), and the parasite indices, spleen indices and size of the average enlarged spleen of each treatment group were set down for the pretreatment, treatment, and post-treatment periods. These percentages for the pretreatment period are based on surveys made on or shortly before the day of the first medication. The percentages of the parasite and spleen indices during the treatment and post-treatment periods are based on approximately two to eight, and one to three surveys, respectively. To permit valid comparisons Table 13 includes only those individuals examined in both the pretreatment (base-line) and treatment periods. Owing to mass migrations, it was not feasible to make follow-up surveys in experimental group 4 (Figure 13 and Table 11) which was treated for only 15 weeks. As a consequence only 149 of 404 individuals who received chloroquine for two to fifteen treatments (bottom of Table 13) had their spleens examined in the follow-up period. However, in the chlorguanide group, 258 of 422 were given post-treatment examination which is more statistically significant.

Both chloroquine and chlorguanide effectively suppressed malaria in the dosages used. Compared with the parasite index found during the base-line surveys, there was a marked drop in incidence of parasitemia; in the same period there was a decided increase in the parasite index of the control groups. The parasite indices of the 504 people on the chloroquine regimen decreased from 23.1 per cent in the pre-treatment period, to 5.3 per cent during treatment, and advanced to 9.0 per cent after treatment was terminated. During the same periods the parasite indices of the 582 individuals on the chlorguanide regimen dropped from 25.9 per cent before treatment to 14.6 per cent during treatment, and to 14.5 per cent after treatment was stopped. The parasite indices of the 328 people on the placebo regimen increased from a base-line of 18.2 per cent to 27.4 per cent during treatment, and dropped to 16.9 per cent, near the seasonal level, in the follow-up surveys. During the suppressive tests of both drugs, the parasite frequency was highest in those receiving two to fifteen treatments, and lowest in those taking most of the treatments.

If one compares the two drugs according to the total number of treatments taken, chloroquine was the more effective except for those who received 26 to 30 treatments with chlorguanide where the results are about equal (Table 13). In line with the slow elimination of chloroquine there are indications of a carry-

TABLE 13

Parasite and spleen indices of the combined groups on the weekly regimens, arranged according to the number of treatments given

NUMBER OF TREATMENTS TAKEN	CHLOROQUINE REGIMEN				CHLORGUANIDE REGIMEN				PLACEBO REGIMEN			
	Number treated	Pre-treatment survey	Treatment surveys	Post-treatment surveys	Number treated	Pre-treatment survey	Treatment surveys	Post-treatment surveys	Number treated	Pre-treatment survey	Treatment surveys	Post-treatment surveys
Parasite indices												
26 to 30	97	29.8	4.1	8.4	51	25.4	3.3	11.1	25	12.5	32.0	9.0
21 to 25	93	25.8	4.3	11.1	70	27.1	14.2	8.7	29	24.1	48.2	26.0
16 to 20	80	28.7	3.7	8.3	51	25.4	9.8	7.8	50	22.0	52.0	25.6
Sub total	270	28.1	4.0	9.3	172	26.1	9.8	9.2	104	20.1	46.1	21.4
11 to 15	56	19.6	1.0	3.3	66	37.8	19.6	37.1	44	15.9	31.8	13.3
6 to 10	84	20.2	8.5	16.6	150	26.6	16.6	12.9	71	8.4	14.0	11.1
2 to 5	94	13.6	3.1	3.5	194	21.1	15.4	15.3	109	21.7	13.0	14.8
Sub total	234	17.4	6.8	8.5	410	25.8	16.5	24.0	224	17.4	18.7	12.9
Grand total	504	23.1	5.3	9.0	582	25.9	14.6	14.5	328	18.2	27.4	16.9
Spleen indices												
16 to 30	255	30.3	25.4	32.5	164	21.7	23.7	24.3	103	44.0	34.9	53.1
2 to 15	149	17.7	12.7	16.8	258	24.5	20.9	23.6	135	13.7	25.1	32.4
Total	404	25.0	20.7	28.0	422	23.7	22.0	24.1	238	22.8	29.4	42.3
Change in the size of the average enlarged spleen												
16 to 30	255	.55	.39	.46	164	.35	.30	.34	103	.76	.68	.92
2 to 15	149	.28	.18	.25	258	.38	.31	.35	135	.15	.46	.56
Total	404	.48	.31	.38	422	.37	.31	.34	238	.33	.55	.70

over effect into the follow-up period as expressed by less malaria during this period as compared to the corresponding period in the controls.

The effect of chloroquine and chlorguanide on the spleen index was not conspicuous. During the period of observation the spleen indices of the groups remained high or increased. In the chlorguanide-treated groups there was no significant change in spleen size, while the spleen size of the chloroquine-treated group appeared to follow the same trends as the parasite indices. In the treatment and post-treatment periods the size of the average enlarged spleen was greater in the controls than in either of the treated groups. During treatments the reduction in the size of the average enlarged spleen was more evident among those receiving chloroquine than in those on the chlorguanide regimen.

Effect of chloroquine and chlorguanide on individuals in various age groups. The data from the entire population, treated and controls, were arranged according to the conventional age groups as given by Russell, West and Manwell, 1946, p. 384, to determine the correlation between age and results of treatment (Table 14). Among 1558 people on weekly treatment, about 60 per cent were over 15 years of age. Men outnumbered women about two to one. The combined pretreatment surveys made in 1948–1949 showed that 33 to 45 per cent of the population had malaria parasites in their blood one or more times.

The parasite indices during the pretreatment period were highest in the two- to fourteen-year-old children, lower in individuals over fifteen years of age, and lowest in infants under one year. During the period of treatment with chloroquine the parasite incidence was highest in the five- to nine-year-old children. In men the reduction to 5.3 per cent was not marked, as the pretreatment incidence was only 11.2 per cent. Suppression in all chlorguanide-treated age groups was less satisfactory than in the corresponding groups given chloroquine. All age groups "under the placebo" showed an increase in parasitemia.

The spleen indices do not reveal many marked differences between the age groups. On all regimens the spleen indices of the older children were the highest, and the indices in infants the lowest. During the treatment period, increase in spleen indices was observed in the men, in the five- to nine-year-olds given chloroquine, and in the men and women given chlorguanide. The women in both groups had a reduction in the size of the average enlarged spleen.

Effect of the drugs on species of plasmodia and gametocytes. The species of parasite and the gametocyte rates observed in individuals under each regimen are given in Table 15 for the periods before, during and after treatment. The percentages are based on the total infections during each period.

Before treatment the incidence of *P. vivax* on the three regimens was comparable. The incidence of *P. falciparum* was analogous in the chloroquine and placebo groups, but was somewhat higher in the chlorguanide group. On the drug regimens the incidence of the parasites was appreciably lower than before treatments and during the same time there was a marked rise in the parasite incidence of the placebo group. On all regimens the *P. falciparum* rates were very low in the post-treatment period. These low rates may have been due to reduced transmission at this season of the year; possibly to the effect of the drugs.

TABLE 14

Parasite and spleen indices and the A.E.S. according to age groups of all the people on weekly regimens

AGE GROUPS YEARS	CHLOROQUINE REGIMEN				CHLORGUANIDE REGIMEN				PLACEBO REGIMEN			
	Total people	Per cent positive in periods			Total people	Per cent positive in periods			Total people	Per cent positive in periods		
		Pre-treatment	Treatment	Post-treatment		Pre-treatment	Treatment	Post-treatment		Pre-treatment	Treatment	Post-treatment
Parasite indices												
0–1	36	8.5	3.4	5.0	50	13.9	11.6	22.2	44	8.1	18.1	13.3
2–4	56	34.7	2.5	11.7	83	31.1	25.0	12.0	50	19.1	43.3	28.5
5–9	83	37.6	10.6	8.9	119	28.2	13.9	28.2	89	15.5	34.5	17.7
10–14	65	26.3	1.9	11.4	119	33.9	17.1	16.2	69	27.2	37.2	33.3
Over 15	447	16.3	4.8	4.5	553	22.5	15.5	11.4	347	17.7	25.0	14.4
Men	312	11.2	5.3	3.2	335	19.2	15.3	10.8	204	19.6	23.3	10.6
Women	135	27.7	3.2	6.4	218	27.4	15.7	11.7	143	15.2	22.9	17.5
Total examinations each period.....	687	628	538	337	924	789	683	223	599	514	337	214
Spleen indices and average enlarged spleen												
0–1	26	0*	0	0	33	22.2	0	0	13	0	12.5	30.7
		0†	0	0		.44	0	0		0	0.18	0.38
2–4	43	23.0	17.6	32.3	64	15.3	7.2	4.1	27	50.0	22.2	21.0
5–9		.53	.23	.47		.34	.12	.04		.75	.44	.31
5–9	74	15.5	17.4	25.0	96	26.3	19.4	17.0	66	25.0	26.4	32.5
		.27	.25	.32		.39	.24	.24		.37	.56	.62
10–14	56	32.2	22.7	31.0	104	35.2	28.2	44.1	52	28.5	36.8	40.0
		.61	.27	.48		.44	.40	.55		.42	.57	.62
Over 15	332	23.5	22.5	27.8	393	19.4	24.9	27.3	216	18.0	31.3	27.6
		.45	.36	.40		.19	.24	.43		.27	.58	.73
Men	252	18.4	23.9	30.7	247	19.7	26.7	36.1	120	13.6	32.4	52.5
		.36	.39	.47		.36	.42	.59		.20	.62	.90
Women	100	36.6	19.2	19.1	146	18.7	22.0	18.7	96	23.0	29.6	43.1
		.66	.27	.21		.54	.28	.27		.35	.54	.59
Total examinations each period.....	525	289	411	298	691	361	500	212	384	136	272	181

* Spleen index.

† Average enlarged spleen.

TABLE 15

Effect of the drugs on the species of plasmodia and on the gametocyte rates

SPECIES	CHLOROQUINE PERIOD						CHLORGUANIDE, PERIOD						CONTROL, PERIOD					
	Pre-treatment		Treatment		Post-treatment		Pre-treatment		Treatment		Post-treatment		Pre-treatment		Treatment		Post-treatment	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Plasmodium vivax	57	43.8	13	43.3	26	74.3	75	36.4	36	31.3	34	87.2	36	37.1	46	39.6	26	63.4
Gametocytes	12	21.1	7	53.8	3	11.5	19	25.3	9	25.0	10	29.4	4	11.1	11	23.9	5	19.2
Plasmodium falciparium	56	43.1	9	30.0	5	14.3	106	51.5	67	58.3	3	7.7	42	43.3	47	40.5	9	21.9
Gametocytes	35	62.5	5	55.5	1	20.0	59	55.7	52	77.6	3	100.0	23	54.8	30	63.8	7	77.7
Plasmodium malariae	—		—		—		—		1	0.9	—		—		1	0.9	—	
Mixed infections	3	2.3	1	33.3	—		—		—		1	2.6	2	2.1	2	1.7	—	
Species undetermined	14	10.8	7	23.3	4	11.4	25	12.1	11	9.6	1	2.6	17	17.5	20	17.2	6	14.6
Total infections found	130		30		35		206		115		39		97		116		41	

In all groups, in most of the periods, the average gametocyte rate of the *P. vivax* infection was about 20 per cent and the *P. falciparum* gametocyte rate was over 50 per cent. From September, 1948, to March, 1949, the respective *vivax* and *falciparum* gametocyte rates for these groups were about 19 and 25 per cent. Under treatment with chloroquine the *P. vivax* gametocyte rate increased from 21.1 to 53.8 per cent, and the *P. falciparum* gametocyte rate dropped slightly (Table 15). Under treatment with chlorguanide there was no change in the *P. vivax* gametocyte rate, but the *P. falciparum* rate rose from 55.7 to 77.6 per cent. Van Goor and Lodens (1950) report a comparable rise in gametocytes found during chlorguanide treatment. However, in the placebo group there were game-

TABLE 16

Number of positive examinations in each of the groups on weekly treatments

GROUP	TOTAL EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE	NUMBER OF TIMES EACH INDIVIDUAL WAS POSITIVE					NUMBER OF POSITIVE EXAMINATIONS
				1	2	3	4	5	
Chlorguanide regimen									
1	186	17	9.1	14	3	—	—	—	20
2	60	8	13.3	7	—	—	—	1	12
3	132	12	9.0	8	4	—	—	—	16
4	305	74	24.2	54	13	5	2	—	103
Total	683	111	16.2	83	20	5	2	1	151
Chloroquine regimen									
1	365	16	4.3	14	1	—	1	—	20
2	33	0	0	—	—	—	—	—	0
3	113	9	7.8	9	—	—	—	—	9
4	27	2	7.4	2	—	—	—	—	2
Total	538	27	5.0	25	1	—	1	—	31*
Grand total	1,221	138		108	21	5	3	1	182

* Does not include two examinations showing two double infections in one person.

tocyte increases also, which were highest for *falciparum* in the follow-up period. About half the slides with *falciparum* crescents also had ring forms.

Parasitemias occurring during the treatment period. During treatment some of the individuals (Table 16) seemingly did not immediately respond to medication in that they remained parasite-positive through two to five treatments before negative smears were obtained. Among the 111 parasite-positive treated individuals on the chlorguanide regimen, there were 151 positive blood slides. Among the 27 cases of parasitemia that occurred during treatment with chloroquine, there were 31 positive slides. In one person of the latter group two examinations revealed double infections with *vivax* and *falciparum* parasites.

A parasitemia with concomitant clinical symptoms is often regarded as the

only acceptable criterion of a break-through of malaria. For obvious reasons the presence of symptoms indicative of clinical malaria, or of sexual forms only (crescents) in the blood indicate questionable break-throughs. Vague symptoms may be due to other diseases, while crescents often remain in the blood long after recovery from clinical *falciparum* malaria. One may also speak of parasitological break-throughs. Because it was impossible to spend more than one or two days a week at each locality in which the population was under treatment, records of chills, fevers and other malaria symptoms that occurred are incomplete.

In the chlorguanide-treated group seven parasitological break-throughs occurred; six with *falciparum* malaria and one undetermined. One break-through occurred in the chloroquine-treated group; it was a mixed *vivax* and *falciparum* infection. After varying intervals without weekly treatments, many individuals, especially those on the chlorguanide regimen, developed overt clinical attacks which cannot be considered as break-throughs. It is noteworthy that only two such cases were observed in the chloroquine groups. However, due to the short periods of observation at each site these cases may not represent a fair sample of the number of overt cases. Although the patients' statements regarding chills and fevers were recorded, we were reluctant to accept them as evidence of overt malaria, even if they were also parasite-positive. Since the observations are incomplete, the few break-throughs observed are not described in detail.

Species of plasmodia found at various intervals during treatment with chloroquine and chlorguanide. The species of parasite found on smears made during the course of each drug program was determined in order to ascertain the effect of the number of treatments on the two principal species of plasmodia (Table 17). The parasitemias are placed in three major categories according to the interval elapsing between the last medication received and the discovery of parasites. An interval of one week indicates that no treatments were missed. An interval of two weeks signifies that one treatment was missed, and so on. The percentages indicated under each category show the number of treatments the average individual received from the time of the first medication to the time parasitemia was discovered. The identified and undetermined species under each category are enumerated in relation to the weekly administrations of drugs received prior to the appearance of parasitemia.

Certain trends in the distribution of the parasitemias apply to both regimens. As the number of treatments increased, there was a decrease in the number of parasitemias discovered. Two-thirds of the total (183) occurred before the fourth treatments were administered. Under each drug regimen only three individuals were parasite-positive after two-thirds (20) of the treatments had been given.

Six treatments with chlorguanide gave results comparable to one treatment with chloroquine, for with the latter a single treatment was followed by 31 parasitemias (two were double infections), and with the former 33 parasitemias occurred after the sixth treatment.

The slower action of chlorguanide may account for the larger number of parasitemias appearing during the chlorguanide regimen. However, when a sufficient number of weekly treatments was taken, the suppressive action of chlorguanide

TABLE 17

Classification of the parasitemias under suppression and effects of the number of treatments on the species of plasmodia

WEEKLY ADMINISTRATION OF DRUG PRIOR TO PARASITEMIA	TIME INTERVAL BETWEEN LAST MEDICATION AND PARASITEMIA									TOTALS
	1 week			2 weeks			3 or more weeks			
	Average number of treatments before parasitemia									
	75 per cent			68 per cent			54 per cent			
	P. vivax	*P. falciparum*	Undetermined	*P. vivax*	*P. falciparum*	Undetermined	*P. vivax*	*P. falciparum*	Undetermined	
Chlorguanide regimen										
1	3	11	—	—	6	1	8	16	4	49
2	—	8	—	—	3	—	5	14	2	32
3	—	5	—	—	3	1	3	2	—*	14
4	1	11	—	—	—	—	—	2	1	15
5	—	—	—	—	1	—	—	1	—	2
6	—	2	—	—	—	—	2	1	—	5
7	—	2	—	—	—	—	4	2	—	8
8	—	3	—	—	—	—	—	1	—	4
9	—	—	—	—	—	—	—	1	—	1
10	1	2	—	—	1	—	—	—	—	4
11	—	2	—	—	—	—	1	—	—	3
12	—	1	—	—	—	1	—	1	—	3
13	1	—	—	—	—	—	—	—	—	1
14	—	—	—	—	—	—	2	1	—	3
17	—	—	—	—	—	—	1	—	—	1
18	2	—	—	—	—	—	—	—	—	2
20	—	—	—	—	—	—	1	—	—	1
21	—	—	—	—	—	—	1	—	—	1
22	—	—	1	—	—	—	—	—	—	1
Totals.....	8	47	1	—	14	3	28	42	7	150
Chloroquine regimen										
1	1	2	1	—	—	1	—	1	—	6
2	—	—	—	—	—	—	1	1	—	2
3	—	1	—	1	—	1	—	4	—	7
4	—	—	—	—	—	—	1	—	—	1
5	—	—	1	—	—	—	2	—	—	3
7	—	—	—	1	1	—	—	—	—	2
8	—	—	—	—	—	—	1	—	—	1
9	1	—	—	—	—	—	1	—	—	2
10	—	—	—	—	—	—	1	—	1	2
11	1	1	—	—	—	—	—	—	—	2
14	—	—	—	1	—	—	—	—	—	1
15	1	—	—	—	—	—	—	—	—	1
19	—	—	1	—	—	—	—	—	—	1
25	—	—	1	—	—	—	—	—	—	1
29	1	—	—	—	—	—	—	—	—	1
Totals.....	5	4	4	3	1	2	7	6	1	33

* One case *P. malariae* not included here.

appeared to be the same as that of chloroquine. After treatment with chlorguanide, more parasite-postive individuals were found in the category receiving 54 per cent of the treatments than in the one receiving 75 per cent. On the other hand, there were no significant differences between the 54 and 75 per cent categories among those on the chloroquine regimen.

The two major subdivisions of treatment frequencies, one week and two weeks (Table 17), 75 per cent and 68 per cent, may be combined with the 54 per cent category of both drugs for the purpose of comparison. Since the comparisons between the categories are based on the approximate per cent of treatments taken, such a combination in no way alters the conclusion, when all comparisons were made between the 75 and 54 per cent groups.

Chlorguanide as a suppressant was less effective than chloroquine against *P. falciparum* and *P. vivax*. In those persons who took most of the treatments, chlorguanide suppressed *P. vivax* more satisfactorily than when only about one-half of the treatments was taken. Irregular administration of chloroquine appeared to suppress *P. vivax* as well as the most regular treatment with both drugs. However, the number of parasitemias discovered in the groups under the chloroquine regimen is almost too small to be of statistical value.

Chloroquine effectively suppressed *P. falciparum*, regardless of the number of treatments missed. The regularity with which chlorguanide treatments were taken had no pronounced effect on the incidence of *falciparum* parasitemias. Of the 103 *P. falciparum* parasitemias discovered in persons on the chlorguanide regimen, 35 occurred after the third treatment, and of the 11 *P. falciparum* positives found among those on chloroquine, only two were found after the third treatment.

SUMMARY

To find the most suitable location for an antimalarial drug evaluation project, 160 places in 14 states of the Republic of Guatemala were surveyed in 1948 and 1949. Malaria parasites were found in 3,106, or 13.27 per cent of 23,390 blood films taken during the surveys, and splenomegaly was found in 1,474 or 19.41 per cent of 7,694 examinations. The parasite incidence ranged from zero in the highlands to as high as 60 per cent in the lowlands. Correspondingly high spleen indices were also found in the lowlands. The percentages of the species of parasites found were: *Plasmodium vivax* 51.5 per cent, *P. falciparum* 45.9 per cent, *P. malariae* 2.6 per cent, species undetermined 16.8 per cent, and multiple infections 2.0 per cent. The parasite indices were highest in children and women and lowest in men and infants. The spleen indices were highest in the older children. The principal vector of malaria in the regions surveyed was *Anopheles albimanus*.

The trials of suppression by chloroquine and chlorguanide were conducted in six areas on the Pacific coastal plain in southern Guatemala. In each area part of the population was treated with chloroquine, part with chlorguanide, and the remainder served as the control. Four population groups received weekly treatments, and two groups received treatment every two weeks. The

experimental populations consisted of 2,087 people. The individuals on the weekly regimens of chloroquine, chlorguanide and placebo totaled 538, 683, and 337, respectively. In the biweekly treatment groups, 229 received chloroquine, 73 chlorguanide, and 227 served as the control. Mass movements of the population occurring during treatment periods accounted for the uneven numbers available for study in some groups.

With weekly chloroquine treatments, the incidence of malaria was reduced from 23.1 per cent to 5.3 per cent, and increased to 9.0 per cent in the follow-up period. The parasite index in the chlorguanide-treated groups dropped from 25.9 per cent before treatment to 14.6 per cent during treatment, and was 14.5 per cent after treatment was discontinued. In the control group, parasite indices increased from 18.2 per cent at the base-line to 27.4 per cent during treatment, and dropped to a low dry-season level of 16.9 per cent in the follow-up period. Chloroquine treatments appeared to reduce the spleen index and the size of the average enlarged spleen. Chlorguanide did not appear to affect the spleen index, but seemed to cause a slight reduction in the average enlarged spleen. Treatments were about equally effective in all ages.

Chloroquine more effectively suppressed both *P. vivax* and *P. falciparum* than chlorguanide. The gametocyte rate of *P. falciparum* rose from 55.7 per cent in the pre-treatment period to 77.6 per cent during treatment; a corresponding increase was noted in the controls, in which the gametocyte rate was 54.7 per cent in the pre-treatment period, 63.8 per cent in the treatment period, and 77.7 per cent in the post-treatment period. About 50 per cent of the blood films with *falciparum* gametocytes also showed ring forms.

Even in individuals taking treatment irregularly, there was a decrease in parasitemias with the increase in the number of treatments taken. Thus, most of the positives, found in the 151 persons given chlorguanide, and in the 31 persons given chloroquine, were reported in individuals who had received but few treatments. Even a small number of doses of chloroquine rapidly and effectively cleared both *vivax* and *falciparum* parasitemias. On the other hand, with *falciparum* at least four doses of chlorguanide were required to obtain results comparable to one treatment with chloroquine.

Biweekly treatments with either drug were not as effective as weekly treatments.

Acknowledgments

Because of the nature of these investigations, we are indebted to many individuals and we take this opportunity to express our thanks: to Dr. Bernado Aldana, Minister of Health and to Dr. Luis B. Galich, Director General of the Public Health Service of Guatemala, who kindly made arrangements for us to conduct these field trials; to Dr. Carlos Tejeda, Surgeon General of the Army of Guatemala for his generosity in providing laboratory space in the Military Hospital in Guatemala City; to the U. S. Air Force for transporting our personnel and supplies and for providing living and laboratory quarters at the San Jose Airbase; to the Misses Marjorie Melton and Elizabeth Guinn and Junior Sanitarian Richard E. Peckham of the U. S. Public Health Service for assistance in the field and for examining the blood films in the laboratory; to Sanitary Engineer Director W. H. W. Komp of the U. S. Public Health Service for suggestions and criticisms regarding the field work, and for

reading the manuscript; to Drs. Paul F. Russell and Wilbur G. Downs of The Rockefeller Foundation, Dr. David Price of the U. S. Public Health Service and Dr. Paulo C. A. Antunes of the Pan American Sanitary Bureau for valuable suggestions during the progress of the work; and to Mr. Marvin Schneiderman, statistician of the Biometrics Section, National Institutes of Health, for his aid in the analysis of the data.

We wish to express our thanks also to the E. I. du Pont de Nemours & Company for supplying the chlorguanide used in these studies.

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A CASE OF VISCERAL LEISHMANIASIS IN SAUDI ARABIA

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Visceral leishmaniasis is a disease relatively common in tropical and subtropical countries. It is present in several Near and Middle East countries, but, as far as we have been able to ascertain, the only reference to its occurrence on the Arabian peninsula are those of Phillips (1904) and of Sarnelli (1933). Phillips refers to two patients with splenic punctures positive for *Leishmania donovani* during hospitalization in Cairo. One of them had lived in Yemen and the other at Mecca. Sarnelli, in a paper on mucocutaneous leishmaniasis, refers to two cases of the visceral form seen in Sana'a Hospital, Yemen.

It may be of some interest to record a case of visceral leishmaniasis which came under observation and which was treated in the Hospital of the Arabian American Oil Company at Dhahran in northeastern Saudi Arabia. This is not because of any particular clinical aspect but because it seems to be the first to have been reported from this region. The finding of the disease in this area is consistent with the general climatic, geographical and ecological characteristics of its distribution elsewhere. It is believed that if previous records do not exist, it is only due to the fact that until very recently the population did not receive much medical assistance and has been out of touch with current progress in medical science. This applies not only to visceral leishmaniasis but to many other diseases which are endemic here.

The area in which the case has been detected is in the Aramco operation zone near the coast of the Persian Gulf. It is an arid desert of sand and rocks with minor elevations. With the exception of very scanty desert growth, the vegetation and water supplies are strictly limited to scattered oases where the settled population lived before the advent of oil exploration. Nomad Bedouins spend a migratory life in the desert moving with their camels according to the availability of water and grazing for their livestock.

There are only recent official records of climatic conditions in the area. Data collected during a period of more than three years indicate average annual temperatures of 78°F., with average high and low annual temperatures of 90° and 69°F. respectively. The highest average monthly temperatures occur from June to September, with values of 102° to 107°F.; the lowest from December to February with values of 75° to 80°F. Maximal daily temperatures reach 127°F., minimal 34°F. Relative humidity reaches extremely high values in late summer, 80 to 90%, with minima of about 40% in late spring and early summer. Average yearly rainfall is rarely more than 1 inch, and it almost never rains from April to November. North and north western winds are predominant throughout the year, increasing in intensity during the period February to June.

CASE REPORT

On October 20th, 1951, a Saudi Arab of about twenty-two years of age was brought to the out-patient department in Dhahran Health Center with an ex-

tensive ulcer involving tongue and mouth. No detailed history was obtainable. He stated that the ulcer had started about fifteen days before and that he had not been feeling well for about one month during which time he noticed a progressive distension of the abdomen. Systemic inquiry was negative, past history non-contributory. On first examination a noma was suspected, and the patient was admitted to the ward because of his very poor general condition.

Physical examination revealed the following findings: markedly emaciated and dehydrated young adult Arab with slight greyish discoloration of the skin. An offensive odor emanated from his mouth and the tongue could not be protruded to its full extent. In the anterior fourth of the tongue, on the left side, an ulcer was present, black in color and surrounded by a white border and again by an erythematous area. Similar ulcers involved the gums and the inner aspect of the cheek; poor dental hygiene. Left eye: bulbar atrophy due to previous extensive keratitis and perforation. Cervical lymph nodes were not palpable. Chest: harsh breath sounds in both upper halves; numerous râles in lower halves. Heart: nothing abnormal except tachycardia; dicrotic pulse. Abdomen: superficial scars due to recent counter-irritations with hot iron in left upper quadrant. The liver was palpable, not tender. The spleen extended past the midline and below the umbilical line to the right lower quadrant, very firm and with hardened edges. Temperature 102.2°F., pulse 124, respiration 28, blood pressure 96/54, weight 74 pounds.

The diagnosis of visceral leishmaniasis was considered because of the marked splenomegaly with fever, emaciation, and mouth lesions.

Blood studies revealed: red blood cells, 2,600,000; hemoglobin 5 Grams per cent; white blood cells 6,200; neutrophils 32%; lymphocytes 54%; monocytes 14%; serum albumin 2.79 Grams per cent, serum globulin 3.21 Grams per cent; Formo-gel test positive. Blood, Kahn negative. Urinalysis revealed proteinuria, microscopic hematuria and cylindruria. In the feces, ova of *Trichuris trichiura* and *Ascaris lumbricoides* were found. Repeated smears for malaria parasites were negative. Marrow smear from sternal puncture stained with Giemsa revealed the presence of *Leishmania donovani* confirming the diagnosis.

Due to nonavailability of pentavalent antimony preparations, intravenous treatment with antimony and potassium tartrate, 3 cc. in 1,000 cc. of 5% glucose, was started on the day after admission. On the next day, however, this was discontinued and Neo-stibosan (ethylstibamine) 0.2 Grams was given intravenously, followed by 0.3 Grams every second day for a total of 4.4 Grams in 28 days. Together with specific treatment, the patient received penicillin, glucose, amino acids and general supportive and symptomatic treatment. During the first five days in the hospital, alimentation via nasogastric tube was adopted due to the incapacity of taking anything by mouth.

Soon after beginning treatment, the general condition of the patient improved. On November 6, the spleen was palpable to the left of the midline and continued to reduce gradually in size. No changes were noticed in the size of the liver. The patient ran an irregular low grade fever which reached a maximum of 103°F. at the end of the treatment on November 11.

On November 21, he developed an inflammatory process in the upper left

maxilla and palate. This developed into necrosis of the bone which sequestrated on December 11, leaving a clean surface. His general condition which had markedly ameliorated during the treatment with Neo-stibosan was compromised, and treatment with blood transfusions and supportive measures was reinstituted. After this, the patient showed a continuous and progressive improvement so as to warrant his discharge from the hospital on January 10, 1952. He was seen again in the clinic on January 21, 1952, and on this date showed no clinical signs of activity of the disease. He had gained twenty-two pounds and his spleen was markedly reduced. He has not reported to the clinic since, but we have been informed that up to the present time (October 1952) the patient is in good health and is leading a normal and active life.

EPIDEMIOLOGICAL INFORMATION

On admission the patient had stated that his home was in Mukhmam, a village in the Qatif Oasis, where he had spent all the twenty-two years of his life, with the exception of short trips to the surrounding villages in the same oasis. He earned his living by farming on a small scale and selling his produce. His only reason for coming as far as Dhahran (26 km.) had been the necessity of seeking medical assistance at the hospital.

On November 27, 1951, a visit was made to his village. Mukhmam is a small village in Qatif Oasis near its western border, only three kilometers from the Dhahran-Ras Tanura road. It consists of a central portion of houses built with stones with typical Arab architecture surrounded by numerous thatched houses. Other habitations are scattered in the gardens in proximity to the village. The population was estimated to be approximately 500–600 people in the village with a few hundred more around it. No adequate information is available about morbidity and mortality in the area. Qatif Oasis is known to be an area of hyperendemic malaria and an intensive program of control with DDT spray has been carried out by the Preventive Medicine Section of the Aramco Medical Division in cooperation with the Saudi Arab Government. The results have been very satisfactory. Mukhmam itself had not been surveyed for malaria due to the fact that it is relatively small and away from the normal routes of traffic. Only a few foreigners are known to have visited the village. DDT spray had been applied to all inside surfaces of all dwellings, stables and other shelters only twenty days prior to our visit and about a week after the patient reported to the clinic. Previous DDT applications in Mukhmam were made in the fall of 1948 and 1949, but not in 1950. Thus, except for the very recent DDT treatment, the village had not been sprayed for two years at the time the patient reported ill.

The patient's home was a thatched house of palm fronds. In the rear was a stable with a donkey, a dog, and several chickens. The ground of the stable was moist with manure kept in a corner to be utilized as fertilizer. A search for *Phlebotomus* was negative, notwithstanding the almost ideal conditions of light, temperature, abundance of interstitial space in walls and roofs, the presence of organic matter in decomposition and the proximity of human and animal quar-

ters. *Phlebotomus papatasii* (Scopoli) has been reported from Hofuf, a similar oasis area about one hundred miles south of Mukhmam. Absence of *Phlebotomus* here may be accounted for by the hour in which the survey was made (morning) and by the recent application of DDT. About fifty dogs were living in the village. It was not possible to examine any of them, and no information was obtained about morbidity or mortality among them. The purpose of the visit was only to ascertain the local conditions surrounding the patient. It was not intended to conduct a complete survey, but only to collect preliminary data which could warrant further investigation. A certain resistance of the population due to fear and suspicion of strangers also had to be considered. The examination was, therefore, limited to only a few children who, as always, were less reluctant. No attempt to collect any specimens was made. About one-third of the children examined had enlarged spleens, but this may have been caused by the hyperendemic malaria in the region. There was no sewage system; the water was obtained from wells outside the gardens and from there brought to the village by a stone aqueduct. It was then collected in large open cisterns from which it was taken by dipping buckets by hand.

CONCLUSION

The clinical diagnosis of visceral leishmaniasis made on the patient and later confirmed by finding of the parasite and by response to specific treatment proves that visceral leishmaniasis exists in the Qatif Oasis, this case being the first one to be reported from this part of Saudi Arabia.

As often seen in the epidemiology of visceral leishmaniasis, the area from which the patient came is an area of malaria hyperendemicity with parasitic and splenic indices reaching (before recent control programs) values of 94 per cent and 72 per cent respectively. No statistics or records are available to indicate the presence of previous leishmaniasis cases. This may be explained by the fact that malaria was a common ailment and in the past received practically no treatment. Under such conditions, cases of visceral leishmaniasis could well have escaped recognition. It is anticipated that more cases may be found in the future, even though the malaria control programs with the use of DDT spray may well interfere with possible vectors of the disease.

SUMMARY

A report is given of a case of visceral leishmaniasis diagnosed and treated in Dhahran Hospital, northeastern Saudi Arabia, the first autochthonous case from Saudi Arabia to be reported in medical literature.

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NATURALLY ACQUIRED YELLOW FEVER IN WILD MONKEYS OF COSTA RICA[1]

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In April 1951 the authors of this report agreed to collaborate in a study of liver and kidney lesions in wild monkeys found dead or dying during the anticipated progress of sylvan (jungle) yellow fever in Costa Rica. At that time the wave which originated in eastern Panama late in 1948 had moved westward across the Isthmus through the Atlantic rain forest and was about to involve eastern Costa Rica. Vargas, directing the Public Health Service network of Costa Rica from San José, and in constant telegraphic contact with all sanitary inspectors throughout the Republic, alerted the people to report promptly the localities where dead monkeys were noted. These reports were immediately investigated by personnel of the Costa Rica Public Health Service. The result has been the acquisition of specimens from thirty-five dead, dying or sick monkeys during a fourteen month period (24 July, 1951–29 September, 1952). Twenty-four of these are considered to exhibit the liver lesion of yellow fever. An additional positive specimen was obtained from a dead monkey in the Isthmus of Rivas, Nicaragua, on 18 July, 1952.

The pathologic diagnoses were made at the Board of Health Laboratory, Ancon, Canal Zone. Most of these diagnoses are based on typical microscopic patterns. A few are based on the scattered distribution of remnants of acidophilic material (Councilman bodies) resisting varying degrees of hepatic autolysis in carcasses undergoing putrefactive changes. Such resistance of the acidophilic material to autolysis represents a phenomenon rarely encountered except in livers altered by the yellow fever lesion, and is quite distinctive in character.

This survey is supported by a concurrent study of the livers of 150 arboreal and semi-arboreal mammals and marsupials, mostly primates, from a collection of 500 animals acquired by Clark (1952) and presented to the Board of Health Laboratory, Ancon, Canal Zone, for histologic examination. In this control group, however, the liver specimens were obtained from autopsies performed immediately after death on shot animals and consequently exhibit no autolytic changes, but they do provide a basis for a comparison as normal livers, involved only in parasitic infections such as liver flukes in bile ducts and microfilariae in blood channels.

[1] The authors wish gratefully to acknowledge the assistance rendered by the sanitary inspectors, local authorities, yellow fever vaccination squads, and all the citizens of Costa Rica who cooperated so effectively in obtaining the specimens from the monkeys and transmitting them to the Ministry of Health in San José.

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Control studies on autolytic changes in normal mammalian livers were conducted by sacrificing six normal rabbits and taking a liver specimen from each successively for six days after death as the carcasses underwent putrefaction.

The Costa Rican monkeys were obtained in localities where epizoötic outbreaks of yellow fever were occurring or anticipated, and many of these localities were simultaneously or subsequently the scene of an epidemic in the human population.

In following up reported epizoötic episodes, positive liver specimens were obtained in only about 10 per cent of the investigations. Frequently either no carcasses could be found or those that were encountered were in too advanced stages of putrefaction to be of use. Shooting of live but apparently sick monkeys secured a number of fresh livers exhibiting typical yellow fever lesions. These investigations not only produced the specimens for this study, but also served as a great stimulus to the vaccination program in rural areas, for they constantly reminded the people of the necessity for vaccination.

HISTORICAL ASPECTS OF THE EPIZOÖTIC PHASE OF SYLVAN YELLOW FEVER

Although some skepticism has been expressed that wild monkeys frequently die of yellow fever, historical evidence and observational studies on the effect of waves of sylvan outbreaks on the arboreal primates have repeatedly indicated that the monkey population has been so involved on numerous occasions. Heretofore, however, with the exception of the detection of the liver lesion pathologically in a marmoset that died soon after capture by Laemmert and de Castro Ferreira (1945) in Brazil, the evidence has been presumptive and not based on pathologic examinations.

Kumm (1951) has consistently held that the arboreal primates, especially howler monkeys, are highly susceptible to yellow fever virus. Laemmert and Kumm (1950) have called attention to one of the earliest references in the literature to the wild monkey as a reservoir for the virus by Balfour (1914), who was impressed by an old saying among the negroes of Trinidad that they could always tell when there was going to be an epidemic of yellow fever on the island, because prior to its appearance red howler monkeys were found dying and dead in the high woods. Kumm states further that a severe epizoötic among the howler monkeys in the State of Rio Grande do Sul, Brazil, in 1947, caused many hundreds to die; that in the Passos area of the State of Minas Gerais the howlers were wiped out by yellow fever in 1936–1937 so that even ten years later almost no howlers could be found there, although whiteface (*Cebus*) were still abundant; that the outbreak of 1945 at Paracatú in Minas Gerais also hit the howler monkeys very badly. Laemmert, quoted by Kumm, observed that *Callithrix* marmosets also often die during epidemics.

The experience of Gaitan (1938) in Guatemala parallels that of Balfour in Trinidad. While investigating an epidemic in the vicinity of Amates in the province of Izabal in 1920, which in retrospect bore the characteristics of a sylvan outbreak, he was addressed by an 80-year old woman, who said, "You know

what to do about yellow fever, but my husband used to know when it was coming." She told him that on several occasions when the disease had devastated the region in the past he would say, "Get ready, yellow fever is coming," and that he based his warning on the observation that the monkeys were sick and dying in the forest.

The significance of monkey deaths preceding yellow fever epidemics in northern Guatemala has long been appreciated by many observers. In 1901 the annihilation of the monkey population in the provinces of Alta Verapaz and Baja Verapaz produced an ominous silence in the jungle which heralded the great epidemic of 1902, as reported by Sanchez F. (1939).

The recent studies conducted by Collias and Southwick (1952) on Barro Colorado Island in Gatun Lake, past which the channel of the Panama Canal runs, have shown that the clans of howler monkeys inhabiting the island were materially reduced in population by an epizoötic which coincided precisely in incidence with the approach of the contemporary wave of sylvan yellow fever that has since invaded Costa Rica and Nicaragua. They, too, noted a reduction in the numbers of marmosets as well. For the first time, furthermore, it was found that the whiteface (*Cebus*) monkeys outnumbered the howlers (*Alouatta palliata aequatorialis*) on the island. This observation may be associated with the feeding habits of the two species, for the howler monkeys generally feed higher up in the trees than the whiteface monkeys, and consequently are in closer proximity to the mosquito fauna of the forest canopy where the yellow fever virus is commonly transmitted.

Although most of the monkeys reported in this series are howlers, two marmosets are included, and five spider monkeys from the region of southewstern Puntarenas (Golfito-Quepos) where howlers are rarely found and the spider monkey (*Ateles*) predominates. The series also includes ten spider monkeys from Guanacaste (Tables 1 and 2).

PATHOLOGIC ANATOMY

Liver. The liver lesion of yellow fever in the wild New-World monkey is in most cases surprisingly similar to the human liver lesion, especially in howling and spider monkeys. Although two marmosets are included in this series, liver tissue is available in only one of them, and the distribution of the acidophilic (Councilman) material is more focal. Intranuclear inclusions discovered by Torres (1928), and so classically described by Cowdry and Kitchen (1929, 1930) in the polygonal cell nuclei of rhesus monkeys experimentally infected with yellow fever, have been found in about 70 per cent of the Costa Rican monkeys, and a full report on these is now in preparation by Johnson, Vargas and Elton (1953).

Since ideal conditions for the preparation of liver specimens for histologic examination are difficult to attain in field pathology, findings must be based on results obtainable under existing conditions. Ten per cent formalin has been used for fixation rather than Zenker's fluid because of its wider availability. The time interval lapsing between the death of the animal and procurement of the specimen cannot be fully controlled. Hence the effect of autolysis during postmortem changes must also be studied and evaluated.

TABLE 1

Vargas monkeys having liver lesion of yellow fever, Costa Rica, 1951–52

SERIES NO.	EPIZOOTIC AREA	PATH ACCESSION NO.	SPECIES	LOCALITY	WHEN FOUND	COMMENTS
1 A	1	6398	Howler	Moin-Zent (Limón)	24 July '51	(*) Appeared very sick. Shot
1	2	6403	Howler	Pital, (Alajuela)	16 Aug. '51	(*) In general area of Sarapiquí epidemic center
2	2	6404	Howler	Pital, (Alajuela)	16 Aug. '51	(*) In general area of Sarapiquí epidemic center
3	2	6405	Howler	Pital, (Alajuela)	16 Aug. '51	(*) In general area of Sarapiquí epidemic center
5	3	6452	Howler	La Fortuna (Alajuela)	22 Nov. '51	Found by grocer and given to a school teacher
6	7	6453	Marmoset	Puerto Cortés (Puntarenas)	4 Apr. '52	Found at San Buena, about 5 miles northwest of Puerto Cortés. Several dead squirrels noted near Ojochal on slope just north of San Buena on 6 April 1952. Howler monkeys, formerly abundant here, had disappeared
10	7	6457	Spider	Golfito (Puntarenas)	5 Jan. '52	From Salama, just north of Golfito.
11	7	6465	Spider	La Uvita (Puntarenas)	16 Apr. '52	Found dead about 100 yards from coast
12	7	6466	Spider	La Uvita (Puntarenas)	16 Apr. '52	(*) Found about 2 miles northeast of La Uvita. Appeared very sick, and was shot
16	7	6522	Spider	Quepos (Puntaremas)	30 May '52	
18	5	6524	Howler	Dos Quebradas (Guanacaste)	20 June '52	Found at 400 meters (1300 feet) elevation, along edge of mountain
19	5	6525	Howler	Los Mollejones (Guanacaste)	26 June '52	(*)
20	5	6526	Spider	Dos Quebradas (Guanacaste)	26 June '52	Advanced autolysis
21	5	6527	Spider	Montañas Berlín (Guanacaste)	26 June '52	(*) Elevation 400 meters
22	5	6528	Howler	Los Mollejones (Guanacaste)	26 June '52	(*)
24	5	6544	Spider	Miravalles (Guanacaste)	10 July '52	(*)
26	5	6546	Howler	Miravalles (Guanacaste)	10 July '52	(*)
28	7	6548	Howler	Quepos (Puntarenas)	13 July '52	Advanced autolysis
29	5	6549	Howler	Cuipilapa (Guanacaste)	14 July '52	(*)
30	5	6574	Howler	Hacienda Tenorioe (Guanacaste)	6 Aug. '52	(*) ("Died today or late last night")
31	6	6598	Spider	El Real (Guanacaste)	27 Sept. '52	
32	6	6599	Spider	El Real (Guanacaste)	27 Sept. '52	(*) Appeared sick. Shot
33	6	6600	Howler	El Real (Guanacaste)	29 Sept. '52	
34	6	6601	Howler	El Real (Guanacaste)	29 Sept. '52	
35	4	127–52	Spider	Southern part of Isthmus of Rivas, Nicaragua	18 July '52	Not a Vargas monkey. This is monkey found by Dr. Jorge Bosheil-Manrique, its body still warm.

(*) These specimens exhibited intranuclear acidophilic inclusions (Torres bodies).

A study of postmortem autolytic changes in the normal mammalian liver under tropical conditions was made by Takos (1952) at the Board of Health Laboratory, Ancon, Canal Zone. Six normal rabbits were killed and allowed to undergo putrefaction, and a liver specimen was obtained from each successively for a period of six days. In this manner the peritoneal cavities remained closed until the specimens were taken, as they would normally under field conditions if unmolested by scavengers. This study adequately confirmed the impression

TABLE 2

Vargas monkeys with livers not showing yellow fever lesion

SERIES NO.	PATH ACCESSION NO.	SPECIES	LOCALITY	EPIZOOTIC AREA ON MAP (FIG. 1)	WHEN FOUND	COMMENT
4	6451	Howler	Santa Cruz	—	3 Mar. '52	This locality is 35 miles south by west of Liberia in the Isthmus of Nicoya, Province of Guanacaste
7	6454	Howler	La Cruz	4	27 Feb. '52	This specimen tentatively indicates that the region southeast of Area 4 on the Pacific watershed was not involved by the epizoötic in February 1952
8	6455	Marmoset	Platanillal	7	17 Oct. '51	No liver specimen received This locality was the source of widespread reports of dead monkeys in October 1951, but only this specimen was obtained. Located just south of Potrero Grande, Province of Puntarenas
9	6456	Howler	La Cruz	4	5 Feb. '52	This specimen tentatively indicates that the region southeast of Area 4 on the Pacific watershed was not involved by the epizoötic in February 1952
13	6467	Spider	Montañas de Verdún	4	24 Apr. '52	This specimen is of equivocal diagnostic significance, in that Torres bodies are present, but no Councilman necrosis
14	6468	Spider	Montañas de Verdún	4	24 Apr. '52	Found "recently dead" This specimen is of equivocal diagnostic significance, in that Torres bodies are present, but no Councilman necrosis
15	6521	Spider	Quepos	7	30 May '52	Montañas de Silencio in the valley of the Rio Savegre just east of Quepos
17	6523	Spider	Los Mollejones	7	15 June '52	This was an infant, captured alive, whose mother had evidently died in the epizoötic. It died a short time later. Elevation 400 meters
23	6543	Howler	Cañas Dulces	5	1 July '52	
25	6545	Spider	Miravalles	5	10 July '52	This was an infant whose mother was missing
27	6547	Spider	Tierras Morenas	5	25 June '52	

already held that the autolytic changes in yellow fever livers were distinctive in contrast with those occurring in the polygonal cells of normal livers. The following findings in the experimental rabbits were noted at determined intervals after death, using the standard haematoxylin-eosin stain:

24 hours. Liver architecture is well preserved. The nuclei have disappeared from about one-third of the polygonal cells. Some of the remaining nuclei stain well. A bluish granular material is present in some of the cells. There is nothing resembling Councilman bodies.

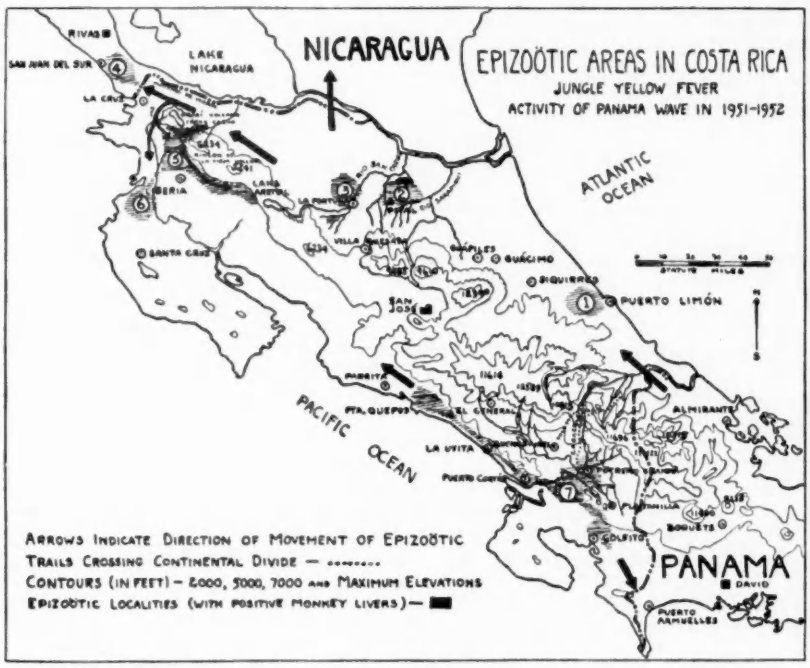

FIG. 1. Interpretation of map showing epizoötic areas in Costa Rica. (Correlate with Tables 1 and 2.)

Area 1. (Pathologic Accession No. 6398). Large numbers of dead monkeys were reported in the vicinity of Zent, just west of Puerto Limón in mid-July, 1951. When this epizoötic was investigated no carcasses remained in satisfactory condition for obtaining liver specimens, hence this monkey, appearing sick in a tree, was shot to obtain the specimen on 24 July, 1951. The first human fatality of the Costa Rican outbreak originated from this general area and occurred also on 24 July, 1951. Earlier reports of monkey deaths in southern Limón, in the Estrella and Talamanca districts, occurring in May and June 1951, were not confirmed.

Area 2. (Pathologic Accession Nos. 6503, 6504, 6505). These specimens, obtained on 16 August, 1951, indicate that the vicinity of Pital on the San Carlos River was involved by the epizoötic at that time. The first human fatality originated here on 19 August, 1951, hence the epizoötic and epidemic phases coincided in this area as they did in Area 1, but occurred about three weeks later. During the period of the epidemics in the San Carlos and Sarapiquí River basins an extensive helicopter reconnaissance was carried out by Captain John R. Peacock, USAF, from 12–25 September, 1951, investigating reports of dead monkeys as well as assisting in the vaccination of the farmers in inaccessible areas. Reliable information was secured that the epizoötic had extended northward to the San Juan River east of Lake Nicaragua.

Area 3. (Pathologic Accession No. 6452). Here near Rancho La Fortuna a dead monkey showing the liver lesion of yellow fever was obtained on 22 November, 1951. Evidently the epizoötic had already swung northward just before it expanded westward to involve the Valley of the Arenal River and the Guatuso region, making La Fortuna a pivot point, for although only about 15–20 miles lie between La Fortuna and Pital, the La Fortuna region

48 hours. The liver is foamy, but much of the architecture has been preserved. All nuclei have disappeared. There are colonies of bacteria in the sinusoids. The cords of polygonal cells are breaking up. The cells are homogeneously and uniformly acidophilic, but there is nothing resembling Councilman bodies.

72 hours. The liver architecture is preserved in small zones only. Polygonal cells are uniformly and homogeneously acidophilic, lack nuclei and have a some-

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had not been involved by the wave in September when the lower reaches of the San Carlos River were active. However, the helicopter reconnaissance had included the La Fortuna region and the valley of the Arenal River as far up as Arenal Lake. Monkeys were reported by the natives as being seen in localities at high elevations (below 2000 feet), where they had never been observed before, although none seemed sick or had been found dead.

Area 4. (Boshell monkey from Nicaragua, Accession No. 127–52, diagnosed by Dr. Leopoldo Navarro Bermudez). In February, March and April, 1952, the epizoötic advanced northwestward from La Fortuna toward the Isthmus of Rivas, following the Atlantic slope of the continental divide. Specimens from La Cruz (Pacific watershed) in February showed no evidence of yellow fever, but the two obtained in the Montañas de Verdun in April, although they exhibit no Councilman changes, are very fresh and do show intranuclear acidophilic inclusion bodies in the polygonal cell nuclei. Since the significance of Torres bodies in wild monkeys remains to be evaluated in a subsequent report, these specimens have not yet been included among the positive group that is based on the demonstrability of Councilman changes alone. If subsequent studies support the diagnostic value of Torres bodies, then the existence of the epizoötic in April 1952 in the Montañas de Verdun, north of Orosí Volcano, will be established.

On 18 July 1952 the Boshell monkey, its body still warm, was found near the Costa Rican border in the Isthmus of Rivas. The liver lesion was classically that of yellow fever.

The region between La Fortuna and the Isthmus of Rivas was very difficult to reach in time to obtain suitable specimens by inspection trips. Repeated reports of epizoötic episodes originated here during the first four months of 1952. In April the fact that there were many dead monkeys in the Montañas de Verdun was readily confirmed, but the carcasses were in such advanced stages of degeneration that specimens could not readily be acquired. Five skulls were collected within 50 square meters in one area, and further to the south only skeletons could be found. At this time the investigator concluded that the *peste* had been moving northward.

It is regretted that the specimens from the Montañas de Verdun must be considered equivocal in diagnostic value, since, if positive, they would support the concept that the epizoötic "turned the corner" north of Orosí Volcano instead of crossing the divide over the 3000 foot contour between Cerro Cacao and Volcán Rincón de la Vieja, as the discussion of Area 5 will indicate.

Area 5. (Pathologic Accession Nos. 6524, 6525, 6526, 6527, 6528, 6544, 6546, 6549 and 6574). Information now available with reference to this involvement of the Province of Guanacaste on the Pacific side of the continental divide indicates that the crossing did not take place at the saddle formed by Arenal Lake as originally anticipated and previously reported by Elton (1952), but implies that it occurred in the region above the 3000 foot contour between Cerro Cacao and Volcán Rincón de la Vieja, for at this point dead monkeys were said to have been noted simultaneously on both sides of the divide, in the upper valley of the Rio Cucaracha on the east and the upper valley of the Rio Ahogado on the west. The epizoötic in the Pacific forest was first detected in the vicinity of Los Mollejones, whence it expanded toward the southeast along the 400 meter (1300 foot) contour, successively involving Montañas Berlin, Dos Quebradas, Cañas, Tierras Morenas, Cuipilapa, and eventually Hacienda Miravalles and Hacienda Tenorio from 15 June to 6 August, 1952, approaching Arenal Lake from the northwest on the Pacific side. The Arenal gap is reported as uninhabited by monkeys.

Since the 2000 foot contour ends a few miles north of this point of apparent crossing of

what liquid appearance. Bacteria are abundant. There are no Councilman bodies.

96 hours. The liver cells are widely scattered and have no orderly pattern anywhere, except for occasional cord-like structure about the central veins. The polygonal cells are acidophilic, but exhibit no Councilman bodies.

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the divide, and although the information available indicates this to be the point where the crossing occurred, speculation as to whether or not the epizoötic actually "turned the corner" north of Volcan Orosí to involve the Pacific forest may still be entertained, especially in consideration of the fact that the northwestern spearhead had attained the southern portion of the Isthmus of Rivas in mid-July, 1952, whence a "blacklash" could have swung southeast around Orosí. This region, however, was "silent" and it must tentatively be assumed that a crossing above the 3000 foot contour developed here.

No human fatalities have as yet been associated with this invasion of Guanacaste, although a few clinically suspicious cases have been reported. Vaccination in Guanacaste had already been completed.

Area 6. (Pathologic Accession Nos. 6598, 6599, 6600, 6601). These specimens were obtained from three dead and one sick monkey in El Real just southwest of Liberia on 27 and 29 September, 1952. The Guanacaste epizoötic area is moving down into the Nicoya Peninsula following the Tempisque River valley.

Area 7. (Pathologic Accession Nos. 6453, 6457, 6465, 6466, 6522 and 6548). Early in October, 1951, dead monkeys were reported in the vicinity of Potrero Grande in southern Puntarenas. At about the same time a positive mouse-protection test was obtained from the blood of a human patient convalescing from clinical yellow fever, who had not been vaccinated and had acquired his infection near Potrero Grande. On 21 October 1951, a human fatality originated here. Subsequently a trail of human fatalities developed to the southeast reaching the Burica Hills Peninsula in February 1952, while another similar trail of human fatalities developed to the northwest, progressively involving Buenos Aires, El General, Barú, Quepos and Parrita as this secondary wave expanded up and down the Pacific forest. According to the sequence of events this secondary wave expanded from a focus in the vicinity of Potrero Grande where the valley of the Cabagra River joins the valley of the Diquis River. The Cabagra Valley is the site of the Cabagra-Talamanca Trail which crosses from the Atlantic to the Pacific watersheds over the continental divide at an elevation of 7000 feet. This seeding of the Pacific forest with the virus seems explainable only by the introduction of virus by a migrant farmer from the Atlantic side, possibly in June or July, 1952, especially since this is probably the most heavily traveled trail crossing the Cordillera in Costa Rica.

The only other route of transmission meriting consideration is a most unlikely one—that the Cabagra Valley was reached by an epizoötic moving from the Pacific side of Panama. A study of the contour lines on the map (Fig. 1) demonstrates that this possibility is most unlikely, for the route of such an epizoötic wave would have involved the vicinity of Boquete, which is thickly populated, and no epidemiologic evidence exists of its presence in either epidemic or epizoötic form in the region from El Volcán (11400 elevation 10 miles west of Boquete) to the coast south of David, all of which is thickly populated and, where not arid open country, is under cultivation. Furthermore Trapido, Galindo and Carpenter (1953) found *Haemagogus spegazzinii falco* Kumm completely absent in this region during the entire rainy season at elevations of 4600 feet.

This study of the epizoötic areas of sylvan yellow fever in Costa Rica is not yet complete, but is apparently approaching its conclusion with involvement of almost all forested areas of the Republic on both sides of the continental divide. The southeastern front of the secondary wave in Puntarenas (Area 7) has evidently failed to advance on the heretofore uninvolved Pacific side of western Panama, and may be considered dissipated by the natural terrain barrier provided by this rather arid and settled region.

120 hours. Blocks and clumps of polygonal cells are widely separated by air spaces. No Councilman bodies are seen.

144 hours. No specimen could be obtained from this animal because of almost complete lysis of the internal organs and a dense infestation of maggots.

This experiment, while not performed on monkey livers, is quite consistent with pathologic experience in respect to postmortem autolysis in general and indicates that the specimens from most of the Costa Rican monkeys must have been obtained within not more than three or four days after death in order to provide the excellent material on which these histopathologic studies are based.

In Figures 2–5 the histology of the liver lesions in the Costa Rican monkeys is presented in representative photomicrographs with individual captions. No illustration of the liver of the Moín (Zent) monkey (No. 6398) is shown since the Councilman bodies are so scarce in this early lesion that it would be difficult to demonstrate them in such a manner without direct examination of the slide. However, Torres bodies are abundant and will be illustrated in a subsequent report. This monkey was shot from a tree where it had been abandoned as a sick member of its clan, and below which, on the ground, was the carcass of a dead monkey in advanced putrefaction.

Histologically the Councilman degeneration consists of a sprinkling of acidophilic hyalin-like material involving the cytoplasm of the polygonal cells. This acidophilic degeneration is much more intense than the diffuse and homogeneous form seen in the polygonal cells of normal liver undergoing autolysis, and contrasts sharply with the uninvolved cells and those involved to lesser degrees. Involvement of entire cells by the Councilman necrosis and vacuolization of the necrosis seem somewhat more prevalent than in the human lesion. In some cases hemorrhagic reactions were marked and a search was necessary to find the Councilman material in which the use of a high power objective helped, and the detection of Torres bodies in the nuclei was consistent with the diagnosis. Up to a certain point postmortem autolysis enhances rather than obscures the contrast, for the Councilman material appears to be highly resistant to the change and is distinctive in character. This accentuated contrast remains distinguishable until only the shadows of the Councilman material remain in the most advanced autolysis before complete disintegration occurs.

FIG. 2. (Accession No. 127-52). Typical yellow fever lesion in liver of howler (Alouatta) monkey. The body of this animal was still warm when found by Dr. Boshell. The changes of post-mortem autolysis are rapidly developing. ×100

FIG. 3. (Accession No. 6404). Typical yellow fever lesion in liver of howler (Alouatta) monkey. Torres bodies are demonstrable as acidophilic inclusions in nuclei of polygonal cells. ×100

FIG. 4. (Accession No. 6526). Typical liver lesion of yellow fever in spider (Ateles) monkey. Post-mortem autolysis is advanced, but contrast between Councilman necrosis and normal cells is enhanced by resistance of acidophilic material to autolytic changes. ×100

FIG. 5. (Accession No. 6453). Yellow fever lesion in liver of marmoset. Distribution of the acidophilic necrosis is midzonal, but also rather focal in that many lobules are not involved and in others the mid-zonal ring of necrosis is not complete. ×100

FIG. 3.

FIG. 5.

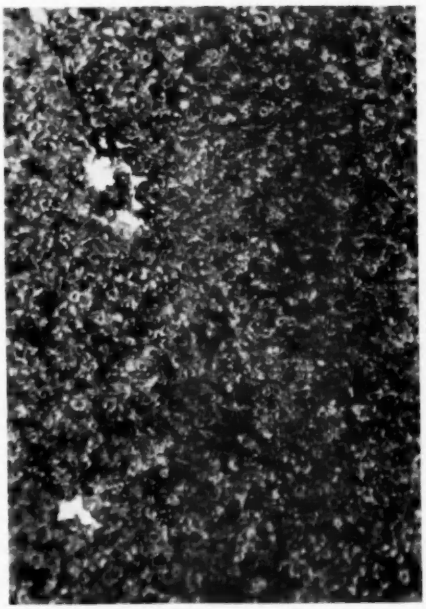

FIG. 2.

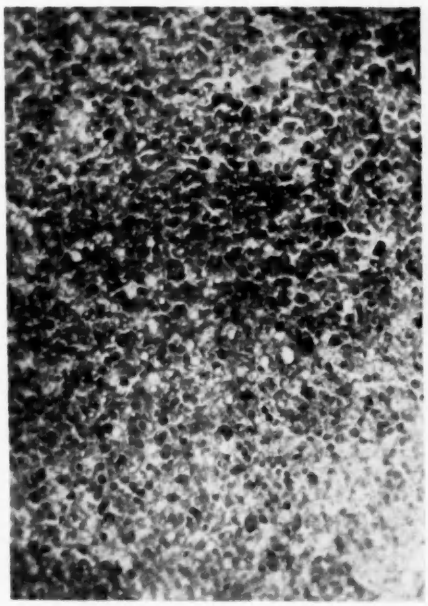

FIG. 4.

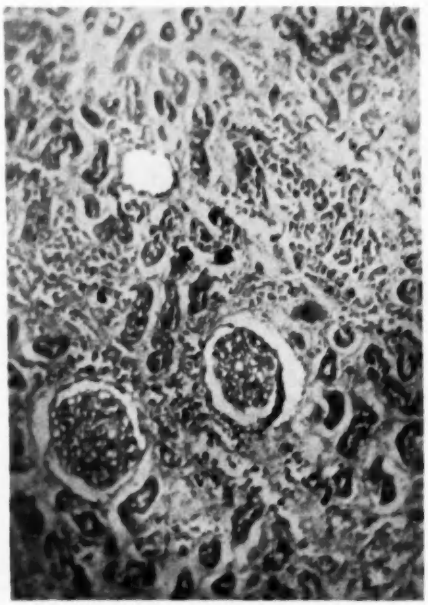

FIG. 7.

FIG. 9.

FIG. 6.

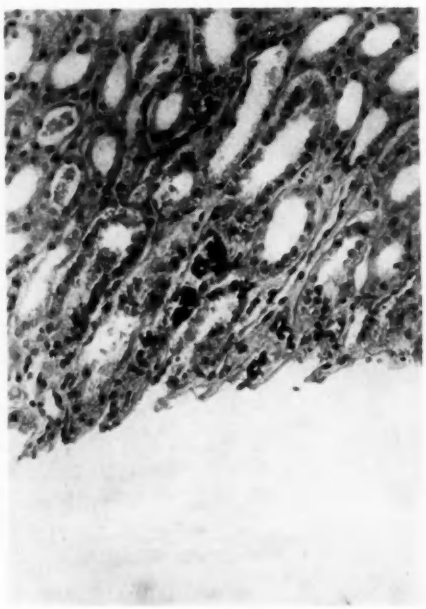

FIG. 8

Kidney. Although the kidney lesion of yellow fever is not pathognomonic as is the liver lesion, nevertheless it contributes materially to the diagnosis. The lower nephron (hemoglobinuric) nephrosis can be even more intense than in the human yellow fever kidney reported by Elton (1952), and the heme casts, like the Councilman material in the liver lesion, resist postmortem autolysis, as shown in Figure 6. The basophilic concretions (Figure 7) in the convoluted tubules, originally described by Councilman (1890), and the basophilic colloid material, also originally described as such by Councilman, and later as "lime casts" by Hoffmann (1929), have all been demonstrable in one or more of the monkey kidneys and also appear highly resistant to autolysis. While not diagnostic of yellow fever in themselves, since these heme casts and basophilic concretions have been seen in association with a number of human liver diseases, especially in carbon tetrachloride poisoning, as reported by Moon (1950), their occurrence under conditions in which yellow fever may be suspected is almost diagnostic especially

TABLE 3

Kidney lesions in Vargas monkeys having yellow fever lesion in liver

SERIES NO.	PATH ACCESSION NO.	SPECIES	LOWER NEPHRON NEPHROSIS	BASOPHILIC CONCRETIONS	COUNCILMAN COLLOID ("LIME CASTS" OF HOFFMANN)
2	6404	Howler	+	−	−
3	6405	Howler	+	−	−
10	6457	Spider	++++	++++	−
29	6549	Howler	−	−	++ In Henle loops.
30	6574	Howler	−	−	++++ In Henle loops

when the hemoglobinuric nephrosis is found in combination with the basophilic concretions, or when the colloid material in the loops of Henle is found alone.

SUMMARY

The acquisition of liver specimens for histopathologic examination from monkeys during the activity of the current epizoötic of yellow fever in Costa Rica

FIG. 6. (Accession No. 6457). Heme casts in collecting tubules of kidney. This demonstrates an anatomically severe form of lower nephron (hemoglobinuric) nephrosis in a spider (Ateles) monkey. The remainder of the kidney structure had undergone advanced postmortem autolysis. ×430

FIG. 7. (Accession No. 6457). Basophilic concretions in convoluted tubules. These correspond to the "crystals" described by Councilman in 1890. They have been found to contain iron and calcium, and are demonstrable in a number of clinical entities involving liver damage, especially in fatal carbon tetrachloride poisoning. ×100

FIG. 8. (Accession No. 6549). Low power view of basophilic material originally described as "colloid spheres" by Councilman in 1890, and again by Hoffmann in 1928 as "Kalkcylin dern", or "lime casts". ×100

FIG. 9. (Accession No. 6549). High power view of same material as illustrated in Fig. 8. This material may be more specifically diagnostic of yellow fever when present than the heme casts and basophilic concretions illustrated in Figs 6 and 7. ×430

has demonstrated that the progress of a wave of the sylvan (jungle) form of the disease can be traced in this manner as well as by similar studies on human fatalities. Out of 35 specimens obtained in Costa Rica, 24 are considered to exhibit the typical liver lesion. It was possible to secure satisfactory specimens in only about 10 per cent of the investigations of the reports of the presence of dead monkeys in the localities involved. An analysis of this experience will be applied to the formulation of a project designed to attempt to check the movement of the wave in the vicinity of La Ceiba, Honduras, in order to prevent involvement of northern Guatemala and Yucatan.

The histopathologic features of yellow fever in the livers and kidneys of the arboreal primates closely parallel those found in man. The Councilman necrosis in the liver, as well as the heme casts, basophilic concretions and colloid material ("lime casts") in the kidney tubules are highly resistant to postmortem autolysis. Intranuclear acidophilic inclusions (Torres bodies) were found in a large percentage of the monkey livers, and an evaluation of their diagnostic significance is now in progress.

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RESPONSE OF THE BABY CHICK TO "B" VIRUS (STRAIN NO. 1)

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The "B" virus (strain No. 1) was isolated from the spleen and central nervous system of patient B (Sabin and Wright, 1934) and has been maintained since the original isolation in 1932 by serial passage in rabbits. The virus is extremely pathogenic for rabbits by most routes of inoculation. Following intracutaneous injection, a hemorrhagic necrotic area develops around the site of inoculation. About 1 to 2 days before paralysis occurs, most rabbits exhibit a tendency to bite the local lesion (Sabin and Wright, 1934). Syrian hamsters respond to the mouse-adapted "B" virus and to the rabbit-adapted "B" virus by the following routes of exposure: intracerebral, intraperitoneal, intradermal, intracardial, intralingual, intratesticular, intramuscular, intranasal, and by rectal instillation (Reagan *et al.*, 1952).

METHODS

The virus employed in this study was the "B" virus (strain No. 1) furnished by the American Type Culture Collection in Washington, D. C. This virus had originally been obtained by the above institution from Dr. Albert B. Sabin of the Children's Hospital in Cincinnati. Before receipt at this laboratory the virus had been passed 67 times in rabbits. The lyophilized rabbit spinal cord of the 67th passage was received at this laboratory and was diluted to a 10 per cent suspension with physiological saline.

EXPERIMENTAL

Three albino rabbits (weight 2 lbs.) were inoculated intradermally with 0.1 cc. of this 10 per cent suspension. The rabbits showed paralysis, with a hemorrhagic necrotic area around the site of infection and nervous symptoms, in 7 days. When symptoms appeared, the rabbits were sacrificed and the spinal cords removed aseptically, ground with alundum and diluted to 20 per cent suspensions with physiological saline. The suspension was then subjected to 5 minutes centrifugation in an angle centrifuge at 2,000 r.p.m. The supernatant fluid was confirmed to contain "B" virus by neutralization tests in rabbits (intradermally). After 6 days the rabbits inoculated with virus and normal serum showed a hemorrhagic necrotic area around the site of inoculation, intense scratching and nervous symptoms, while the rabbits inoculated with virus and immune serum appeared normal. This confirmed the virus to be "B" virus.

Twelve 1-day-old baby chicks (furnished by a local farm in the State of Maryland) from a Newcastle Disease-free flock, were injected in the right frontal lobe with 0.03 cc. of supernatant fluid from the virus-bearing rabbit cord suspension. From 3 to 4 days after injection the baby chicks showed irritability, nervous

tremors and paralysis. When paralysis developed, the brains were removed aseptically, ground with alundum and diluted to a 10 per cent suspension with physiological saline. This suspension was passed to 12 more baby chicks by intracerebral injection. The 'B" virus was carried serially through 6 passages in the baby chick by intracerebral inoculation as shown in Table 1. The virus-bearing brain suspension from the 5th intracerebral baby chick passage was confirmed to be "B" virus by neutralization tests in rabbits intradermally. After 4 days the rabbits inoculated with virus and normal serum showed a hemorrhagic

TABLE 1

Response of baby chicks to "B" virus (strain No. 1)

PASSAGES	NO. CHICKS EXPOSED	NO. CHICKS SHOWING SYMPTOMS	*MIN. TO MAX. INCUBATION PERIOD (DAYS)
1	12	12	3–4
2	5	5	2–3
3	12	12	3
4	8	8	3
5	8	8	3
6	4	4	3–4

* Incubation period = time between exposure to virus and onset of symptoms.

TABLE 2

Response of baby chicks to "B" virus (strain No. 1) of the 5th intracerebral chick passage

ROUTE OF INOCULATION	NO. CHICKS EXPOSED	NO. CHICKS SHOWING SYMPTOMS	*MIN. TO MAX. INCUBATION PERIOD (DAYS)
Intraperitoneal	4	4	3–4
Intrarectal	4	4	3–4
Intradermal	4	4	3–4
Intracardial	4	4	3–4
Intramuscular	4	4	3–4
Intralingual	4	4	3–4
Intranasal	4	4	3–4
Intraoral	4	0	—
Intracerebral	4	4	3–4

* Incubation period-time between exposure to virus and onset of symptoms

necrotic area around the site of inoculation, intense scratching and nervous symptoms, while the rabbits inoculated with virus and immune serum appeared normal. This confirmed the virus from the 5th intracerebral baby chick passage to be "B" virus.

Thirty-six healthy 1-day-old baby chicks were divided into 9 groups of 4 chicks each. Chicks of the 9 groups were administered 0.03 cc. of a 10 per cent suspension of the virus-bearing chick brain of the 5th intracerebral baby chick passage by the following methods: intraperitoneal, intrarectal, intradermal, intracardial, intramuscular, intralingual, intranasal, intraoral, and intracerebral.

After inoculation all groups were observed twice daily for nervous symptoms

characteristically found in baby chicks infected intracerebrally and experimentally with "B" virus. The results of this group exposure, as given in Table 2, show that all the chicks were infected except those inoculated intraorally.

SUMMARY

A strain of rabbit-adapted "B" virus (Sabin and Wright, 1934) has been successfully transmitted intracerebrally to 1-day-old White Leghorn chicks. Virus from a chick-brain suspension of the 5th intracerebral passage was transmitted successfully to baby chicks by the following routes of exposure: intraperitoneal, intrarectal, intradermal, intracardial, intramuscular, intralingual, intranasal, and intracerebral. Baby chicks showed typical symptoms of "B" virus disease between 3 and 4 days after inoculation.

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THE INFLUENCE OF PREVIOUS INFECTION OF MICE WITH *SCHISTOSOMA MANSONI* ON A CHALLENGING INFECTION WITH THE HOMOLOGOUS PARASITE[1]

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Clinical evidence has long suggested the development in human and a few other vertebrate hosts of an immunity to schistosomes following infection with the homologous parasite. Yet adequate experimental proof of the effectiveness of this immunity in the protection of infected hosts against superinfection is lacking. It was the purpose of this investigation to ascertain whether fewer parasites would become established from a second exposure in mice previously infected with *Schistosoma mansoni* than from an accompanying control infection of previously uninfected mice.

MATERIALS AND METHODS

Female albino mice[2] were exposed to 50 cercariae each of *Schistosoma mansoni* from laboratory-bred, infected snails of the Puerto Rican strain of *Australorbis glabratus*. With the single exception noted below these mice weighed between 18 and 20 grams. The procedure was that described by Olivier and Stirewalt (1952), in which the tails of mice were immersed for an hour in Kahn tubes containing the cercariae in dechlorinated tap water.

At varying intervals after the end of the initial exposure—one hour, five hours, one day, one week, four weeks and eight weeks—the mice were given a challenging exposure using the same technique. Concurrently, control mice which had had no previous contact with schistosomes were exposed to cercariae from the same cercarial pool.

When the interval between exposures was four weeks or less the parasites of the initial and challenging infections were tagged by their sex. Half of the mice were exposed initially to 50 male and half to 50 female cercariae. Subsequent exposure, then, was to 50 cercariae of the opposite sex. To provide these cercariae of known sex, the sex of the infection in snails which had been exposed to one miracidium each had been previously established by allowing cercariae collected from them to mature in other mice.

Unisexual infections of this type permitted accurate identification of schistosomes of the original and challenging infections, but they limited the study in that only one sex of parasite was present in the host from the original exposure. Such unisexual infections, of course, produce different pathological changes from

[1] The opinions contained in this paper are those of the author and are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.

[2] The mice were from a stock maintained in the Animal Laboratories of the Naval Medical Research Institute from the Webster strain of CFW mice transferred from the National Institutes of Health, Bethesda, Maryland.

those associated with bisexual infections. It might be expected that the immunological effects of unisexual and bisexual infections would differ also. Therefore it was desirable to give some mice initial bisexual infections and to permit the parasites to mature to the stage of egg production before the challenging exposure was given. The handling of these mice, for which an eight-week interval was allowed between exposures, varied in several considerations from that described above. The initial exposure was presented by partial immersion, one week after birth, since fourteen weeks would elapse before autopsy. To insure egg production, 25 male and 25 female cercariae comprised the initial infective suspension. The challenging suspension was 50 mixed cercariae for both previously infected and control mice.

In order to avoid behavior characteristics peculiar to any one cercarial suspension, the mice studied at each inter-exposure interval, a minimum of 30 experimental and 30 control animals, were handled in sub-groups of ten experimental and ten controls each at various times throughout the experimental period.

Adult schistosomes were recovered and counted at autopsy of the mice six weeks after the challenging exposure. When the parasites of the two infections were tagged by their sex, worm recovery from the initial and challenging infections in the experimental mice was compared with that from the respective control mice. When both the original and the challenging infections were bisexual, the total parasite burden from the experimental mice was compared with the totals collected from the controls for both the initial and the challenging exposures.

Early in the course of the work, when it became evident that a very recent previous infection reduced the number of adult parasites which matured from a subsequent exposure, a method was evolved for evaluating cercarial penetration numerically. This method depended on precise control of the exposures. It may be emphasized here, then, that each mouse was exposed to known numbers of cercariae by tail immersion, since it was imperative to avoid loss of cercariae by splashing, fecal contamination or spreading over a large furred area of host skin. At the end of the hour's exposure the number of cercariae which had not penetrated was recorded for each mouse as follows. Each mouse tail was lifted from the exposure tube and re-immersed several times to rinse off non-penetrating cercariae which might be clinging to it. The mice were removed to cages and marked according to their number on the exposure rack by a spot of dye at the base of the head. Each infective suspension was poured into a small evaporating dish with a ruled bottom, the tube rinsed well, the rinse water added to that in the evaporating dish, and the cercariae counted. This number was subtracted from 50, the exposure density, to give the maximum number of parasites which could have penetrated the mouse. Though it must be remembered that all of these may not have successfully invaded, and that this is the maximum number which could have penetrated, for brevity this will be referred to as the number of penetrating cercariae.

As the experiment neared completion, the need for further information became

obvious. First, it was necessary to find out whether suppression of secondary cercarial penetration in mice recently exposed occurred when handling of the mice or the soaking of their tails in water without cercariae was substituted for the initial cercarial penetration. In other words, was the inhibiting reaction nonspecific, in the sense that it might be a general fear response of the mice related to handling, or a local swelling of skin tissues resulting from soaking the tails in water? Secondly, the question arose whether the phenomenon of inhibition of cercarial penetration was a local one, or whether penetration of other skin than that of the tail by the first cercariae would also inhibit invasion of the tail skin by the cercariae of the second exposure.

To answer the first question, 30 mice in three equal subgroups were handled in exactly the same manner as described above, except that in the initial procedure no cercariae were present in the water in the tubes. The tails of these mice were simply suspended in water for one hour, and five hours later exposed to 50 cercariae per mouse. Control mice were not handled before exposure, but were exposed concurrently with the experimental groups. The number of cercariae penetrating the tails of the experimental mice was then compared with that invading the controls.

To determine whether the inhibition was a local or a general reaction, 30 mice in three equal sub-groups were initially exposed by immersing their feet and bellies in shallow water in large square glass ash trays in such a way that the tails of the mice were taped out of the water along the shallow depression designed for holding a cigarette. The mice were further restrained by a wide adhesive tape across the top of that corner of the ash tray which held the mouse. Twenty-four hours after the mice had been exposed by wading, they were exposed by tail as described above. Controls were not previously exposed or handled. Penetration data were recorded as previously described.

Both the adult parasite yields and the number of cercariae penetrating the experimental and control mice were compared statistically for mathematical significance and graphed.

RESULTS

A brief summary of the results in terms of adult parasite burdens of the mice is given in Table 1. At the interexposure intervals of one hour, one day and two months, the number of schistosomes per mouse developing from the challenging infection was significantly reduced ($p < 0.001$) below that of specific control mice. No such difference was found when the interval between exposures was one week or one month.

The number of adult schistosomes recovered at autopsy from the initial and challenging infections in the experimental and control mice is presented in greater detail in Figures 1, 2, 3 and 4. In these, the accumulated percentage of 50-cercaria samples (ordinate) is plotted against the number of worms recovered per 50-cercaria sample (abscissa). The average number of parasites maturing per 50 cercariae in experimental and control mice (Table 1), together with the ranges (Figs. 1, 2, 3 and 4) may be compared from these sources.

At the interexposure interval of one hour all but one of the 30 challenging experimental 50-cercaria samples produced fewer adult schistosomes than the average for either the control or the initial experimental samples; at the one

TABLE 1

Average worm burden per mouse from the challenging exposure presented at the indicated intervals after the initial exposure and compared with that of control mice

INTERVAL BETWEEN EXPOSURES	DESCRIPTION OF SCHISTOSOMES OF INITIAL INFECTION	WORM BURDEN FROM CHALLENGING INFECTION			
		Experimental		Control	
		No. Mice	Av. No. Worms ± SE	No. Mice	Av. No. Worms ± SE
One hour	Larvae in skin	30	6.6* ± 1.1	30	20.3 ± 1.4
One day	Larvae in skin	28	9.2* ± 1.1	30	17.9 ± 1.5
One week	Larvae migrating	30	19.1 ± 1.3	30	18.0 ± 0.8
One month	Young worms in portal veins	30	15.4 ± 0.9	30	13.4 ± 1.5
Two months	Adult worms and eggs	26	10.7* ± 1.1	27	17.1 ± 1.1

* Statistically significant variation from control (p less than 0.001).

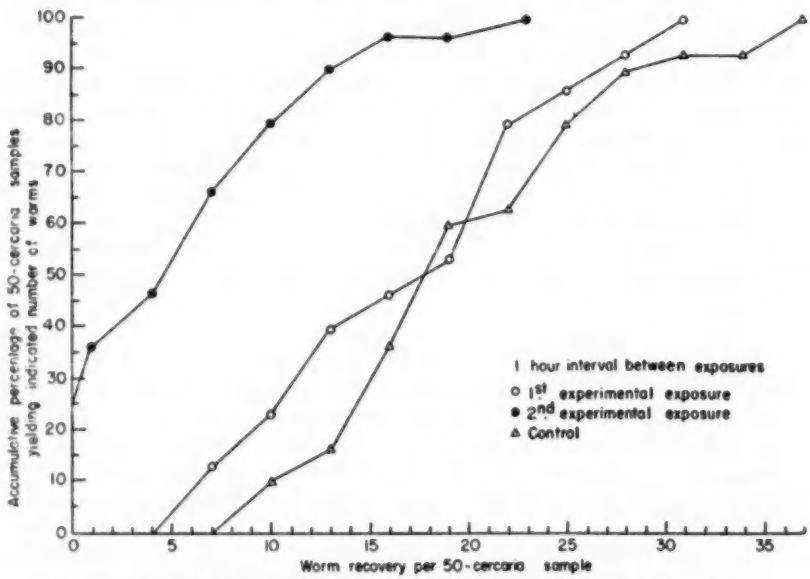

Fig. 1. Accumulative frequency distribution showing percentage of 50-cercaria samples yielding ≡ the indicated number of adult worms in class intervals of 3.

day interval, all but four challenging experimental samples were below the control average.

The general close coincidence of the lines (Figs. 1, 2, 3 and 4) representing worm

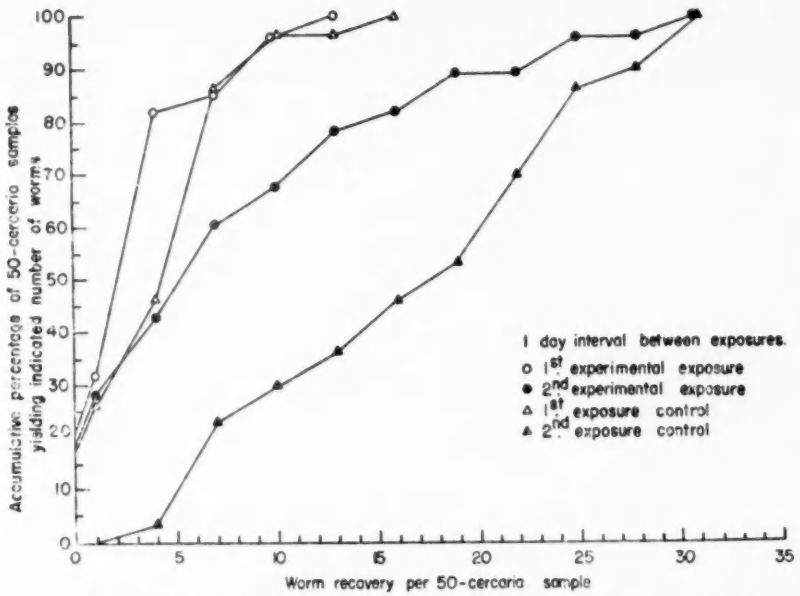
Accumulative percentage of 50-cercaria samples yielding indicated number of worms
Worm recovery per 50-cercaria sample
1 day interval between exposures.
1st experimental exposure
2nd experimental exposure
1st exposure control
2nd exposure control

Fig.2. Accumulative frequency distribution showing percentage of 50-cercaria samples yielding ≦ the indicated number of adult worms in class intervals of 3.

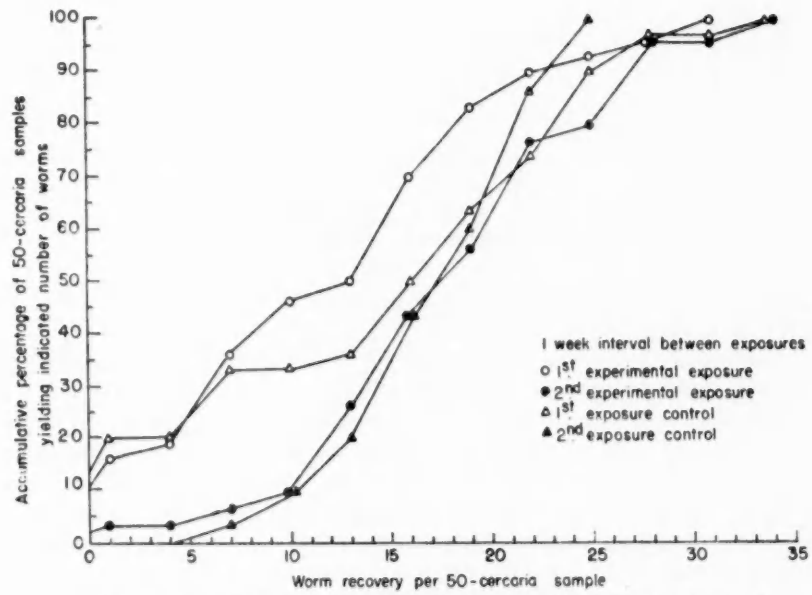
Accumulative percentage of 50-cercaria samples yielding indicated number of worms
Worm recovery per 50-cercaria sample
1 week interval between exposures
1st experimental exposure
2nd experimental exposure
1st exposure control
2nd exposure control

Fig.3. Accumulative frequency distribution showing percentage of 50-cercaria samples yielding ≦ the indicated number of adult worms in class intervals of 3.

yields from the primary experimental exposures and their controls shows that there was no real difference in parasite development between these groups. The fact that these data may differ markedly from the control data for the second infection, however, emphasizes the need for specific control exposures made concurrently under the same conditions and from the same cercarial pool.

When a week's time had elapsed between the two experimental exposures, there was no significant difference between parasite development in the experimental mice and in their controls (Fig. 3). The same agreement between the

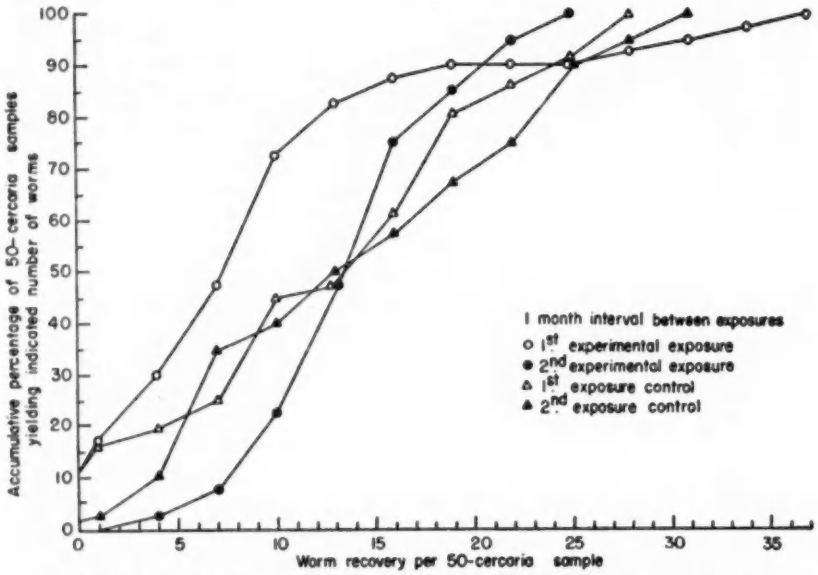

Fig. 4. Accumulative frequency distribution showing percentage of 50-cercaria samples yielding ≦ the indicated number of adult worms in class intervals of 3.

parasite burdens from the challenging exposure and its control was found when the interexposure interval was one month (Fig. 4).

In the above experiments the mice all harbored worms of only one sex when the challenging exposures were presented. Worm recovery from mice which had a mature bisexual infection at the time of presentation of the second exposure is compared with the total control worm recovery in Table 2. Since an interval of eight weeks had elapsed between exposures, adult worms of both sexes as well as eggs enclosing viable miracidia had developed from the initial exposure. A significant reduction in worm burden is shown in the twice-exposed mice, as compared with that from both groups of control mice exposed only once: 21.4 per cent and 34.3 per cent respectively. The fact that the initial exposure was

given when the mice were a week old may be of importance. Attempts failed to demonstrate a similar reduction in the number of parasites from the challenging infection in mice which were adult when they were initially infected.

The technique employed here, which necessitates comparison of the parasite burden from superinfected mice, exposed initially and again eight weeks later, to 50 mixed cercariae, with that from initial and challenging controls exposed only once to equal cercarial densities, limits the conclusions which can be safely drawn. Apparently, however, some individual mice became much more resistant to the challenging infection than others. No hint of the mechanism of resistance was given by examination of the mice at autopsy in that no worms were found dead or walled off in the host tissues. No additional information was given by autopsy of one of the three groups at four instead of six weeks after the challenging exposure, except a suggestion that maturity of the younger worms was somewhat delayed in the superinfected mice as compared with the parasites in the

TABLE 2

Comparison of worm recovery from experimental mice given initial exposure at the age of one week and the challenging eight weeks later with that of their initial and challenging controls combined. Initial exposure of both experimental and control mice was 25 male and 25 female cercariae; challenging, 50 cercariae of both sexes

NUMBER OF MICE		WORM RECOVERY IN PER CENT			
Experimental	Control	Experimental		Control	
		Average	Range	Average	Range
8	10	21.8	14–30	37.0	27–46
10	7	20.9	13–28	32.3	28–36
8	10	21.6	13–33	30.0	21–43

challenging control mice. The wide range usually noted in the rate of growth of schistosomes in mice, however, tends to invalidate observations of this type.

The apparent development of resistance in mice to superinfection when the interval between exposures was one day or less suggested that the mechanism of resistance might be the development of a barrier to penetration of cercariae subsequently presented. Since preliminary studies had indicated this possibility, the number of cercariae which had not penetrated had been recorded at each exposure for each mouse. Therefore, it was possible to calculate the maximum number of cercariae which could have penetrated. These data are given in Table 3 and Figures 5 through 11, according to the same scheme as the figures showing adult worm burdens.

An interexposure interval of one hour was long enough for the development of a partial skin barrier to the cercariae of the second exposure (Table 3 and Fig. 5). Thirty-nine of the 40 samples of the challenging experimental exposure were below the average for either the control or the initial experimental exposure.

At the five hour interval between exposures, also, penetration of cercariae into the mice previously exposed was significantly inhibited (Table 3 and Fig. 6). Twenty-four of the 30 challenging experimental samples fell below the control average.

The results were similar when the challenging exposure followed the initial by one day (Table 3 and Fig. 7). In this case, 78 of the 99 challenging experimental samples were less than the control average.

When two days had elapsed between the two experimental exposures, (Table 3 and Fig. 8) the line representing the challenging experimental penetration still deviates somewhat from its control (Table 3 and Fig. 8), but this deviation is without statistical significance at the 0.1 per cent level. No inhibition of the

TABLE 3

Average maximum cercarial penetration per mouse from the challenging exposure presented at the indicated intervals after the initial exposure and compared with that of the controls

INTERVAL BETWEEN EXPOSURES	DESCRIPTION OF SCHISTOSOMES OF INITIAL INFECTION	CERCARIAL PENETRATION OF CHALLENGING INFECTION			
		Experimental		Control	
		No. Mice	Av. No. Cerc. ± SE	No. Mice	Av. No. Cerc. ± SE
One hour	Larvae in skin	40	36.1* ± 1.1	20	45.2 ± 0.6
Five hours	Larvae in skin	30	29.9* ± 1.7	15	37.8 ± 1.4
One day	Larvae in skin	99	36.1* ± 0.8	50	43.4 ± 0.7
Two days	Larvae in skin	30	32.6 ± 1.3	15	36.9 ± 1.4
One week	Larvae migrating from skin	68	43.2 ± 0.8	35	42.3 ± 1.2
One month	Young worms in portal veins	130	44.9 ± 0.4	47	41.7 ± 0.7
Two months	Adult worms and eggs	46	40.9 ± 0.9	24	41.6 ± 1.1

* Statistically significant variation from control (p less than 0.001).

challenging exposure at all was observed when a week or more was allowed between infections (Table 3 and Figs. 9 and 10).

As may be seen in Figure 11, no statistically significant inhibition of cercarial penetration was produced by merely soaking the tails of mice in water instead of exposing them to primary cercarial penetration. Since the tail-soaking required the same handling of the mice as the initial exposure, the possibility that the barrier to penetration could have been produced merely by previous handling was also thus eliminated.

Examination of Figure 12 reveals that the reaction, in the case of exposure of mice by tail, is a local one. The cercariae of the challenging exposure penetrated the tails of mice recently invaded by schistosomes through other skin areas in as great numbers as they did the tails of control mice with no previous experience with schistosomes.

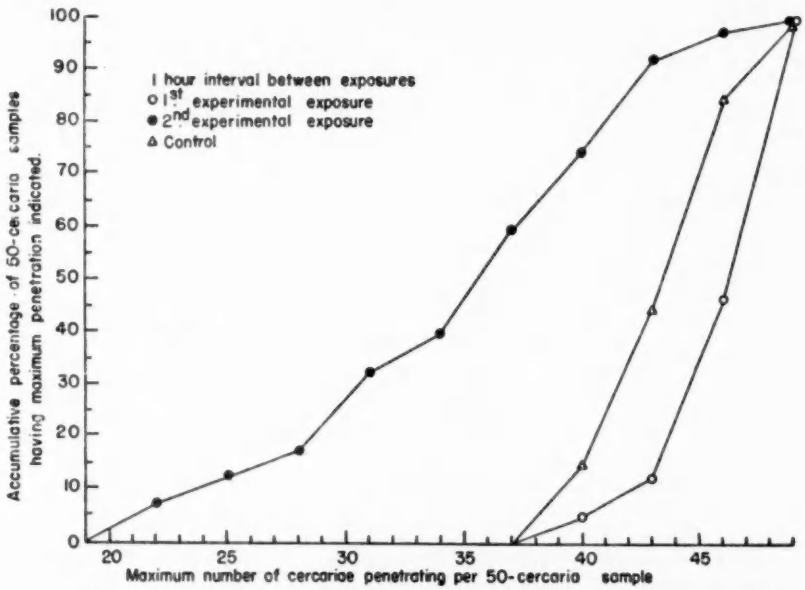
1 hour interval between exposures
o 1st experimental exposure
● 2nd experimental exposure
Δ Control
Accumulative percentage of 50-cercaria samples having maximum penetration indicated.
Maximum number of cercariae penetrating per 50-cercaria sample

Fig. 5. Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was ≦ that indicated. Data are by class intervals of 3.

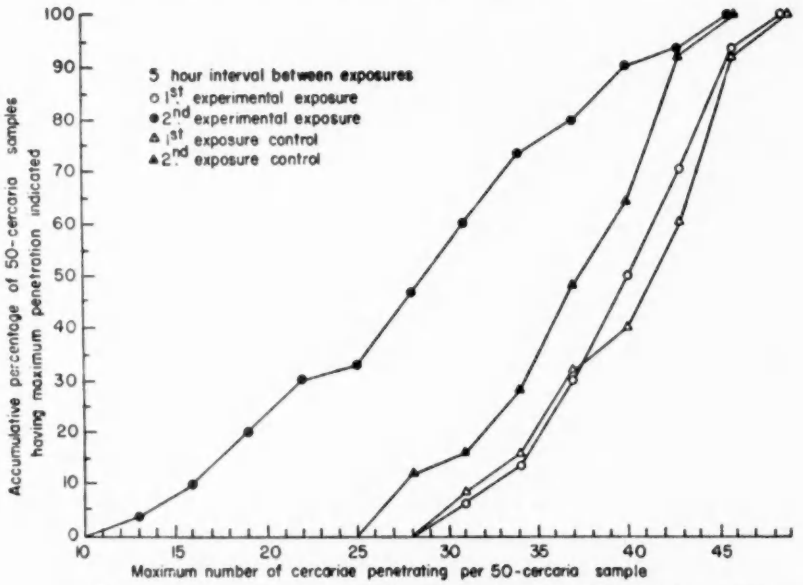
5 hour interval between exposures
o 1st experimental exposure
● 2nd experimental exposure
Δ 1st exposure control
▲ 2nd exposure control
Accumulative percentage of 50-cercaria samples having maximum penetration indicated
Maximum number of cercariae penetrating per 50-cercaria sample

Fig. 6. Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was ≦ that indicated. Data are by class intervals of 3.

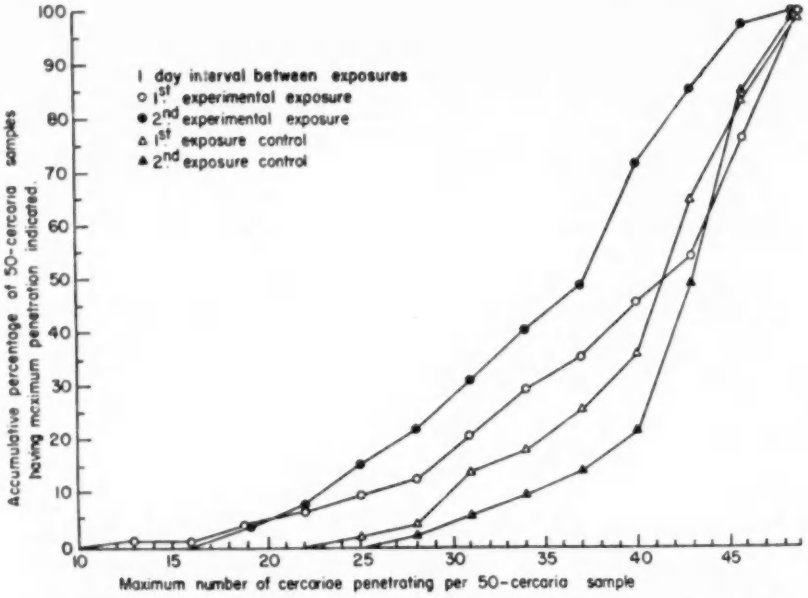
1 day interval between exposures
○ 1st experimental exposure
● 2nd experimental exposure
△ 1st exposure control
▲ 2nd exposure control
Accumulative percentage of 50-cercaria samples having maximum penetration indicated.
Maximum number of cercariae penetrating per 50-cercaria sample

Fig. 7. Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was ≦ that indicated. Data are by class intervals of 3.

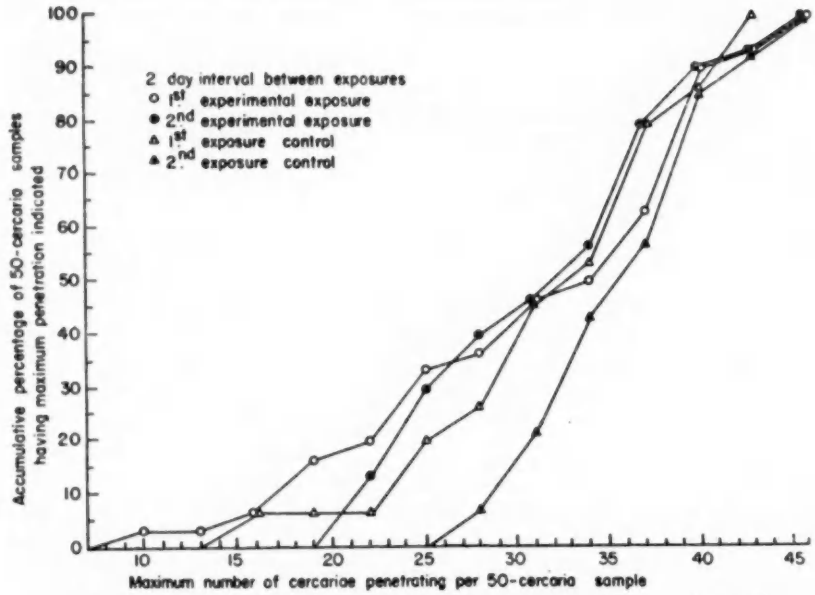
2 day interval between exposures
○ 1st experimental exposure
● 2nd experimental exposure
△ 1st exposure control
▲ 2nd exposure control
Accumulative percentage of 50-cercaria samples having maximum penetration indicated
Maximum number of cercariae penetrating per 50-cercaria sample

Fig. 8. Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was ≦ that indicated. Data are by class intervals of 3.

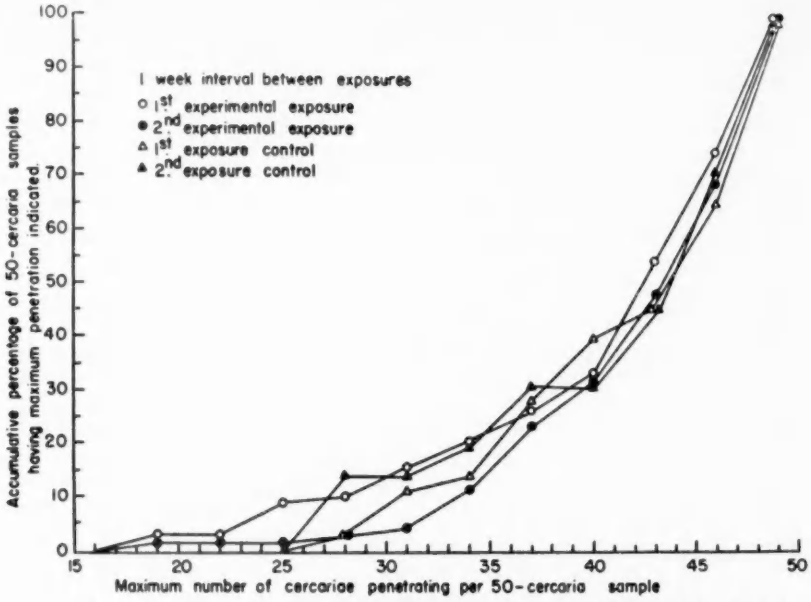
1 week interval between exposures
○ 1st experimental exposure
● 2nd experimental exposure
△ 1st exposure control
▲ 2nd exposure control
Accumulative percentage of 50-cercaria samples having maximum penetration indicated.
Maximum number of cercariae penetrating per 50-cercaria sample

Fig. 9. Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was ≦ that indicated. Data are by class intervals of 3.

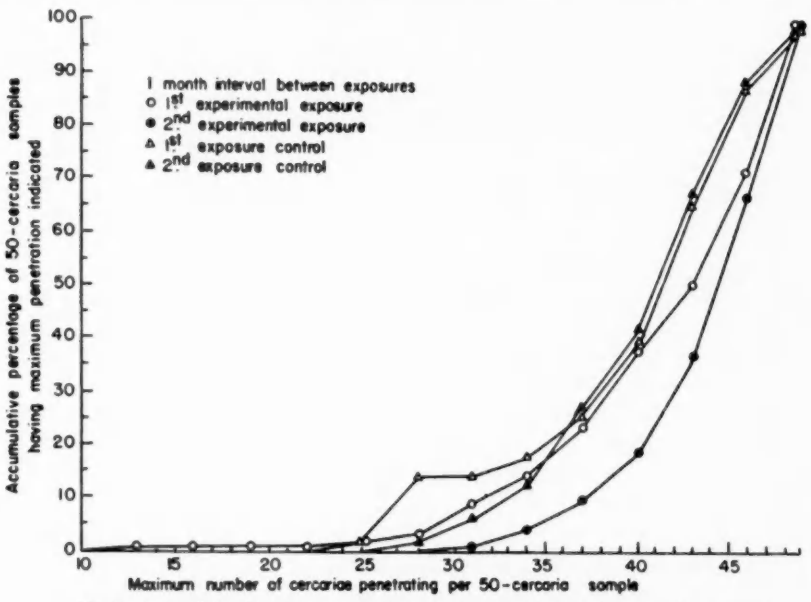
1 month interval between exposures
○ 1st experimental exposure
● 2nd experimental exposure
△ 1st exposure control
▲ 2nd exposure control
Accumulative percentage of 50-cercaria samples having maximum penetration indicated
Maximum number of cercariae penetrating per 50-cercaria sample

Fig.10. Accumulative frequency distribution showing percentage of 50-cercaria samples in which the maximum number penetrating was ≦ that indicated. Data are by class intervals of 3.

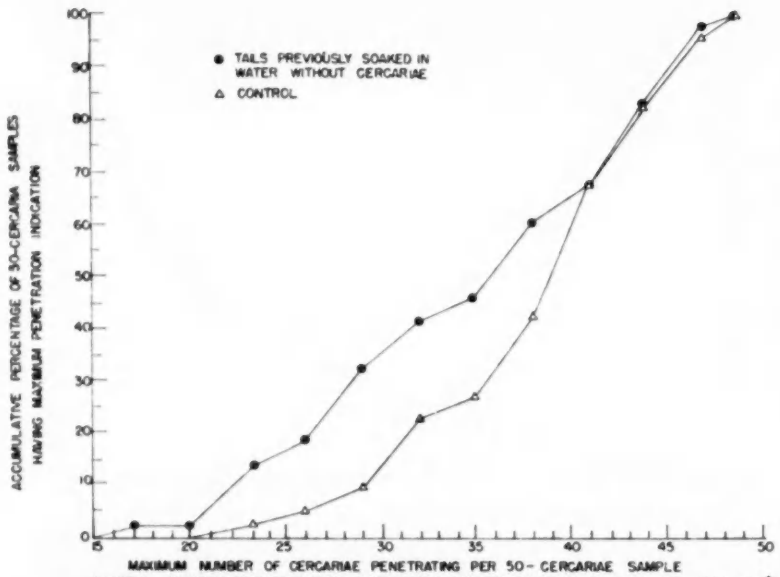
TAILS PREVIOUSLY SOAKED IN WATER WITHOUT CERCARIAE
CONTROL
ACCUMULATIVE PERCENTAGE OF 50-CERCARIA SAMPLES HAVING MAXIMUM PENETRATION INDICATION
MAXIMUM NUMBER OF CERCARIAE PENETRATING PER 50-CERCARIAE SAMPLE

FIGURE 11. ACCUMULATIVE FREQUENCY DISTRIBUTION SHOWING PERCENTAGE OF 50-CERCARIAE SAMPLES IN WHICH THE MAXIMUM NUMBER PENETRATING WAS ≤ THAT INDICATED. DATA ARE BY CLASS INTERVALS OF 3.

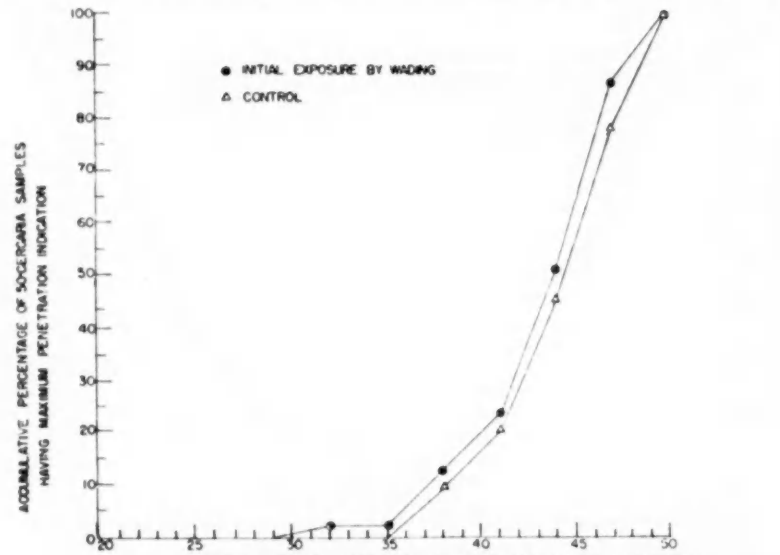
INITIAL EXPOSURE BY WADING
CONTROL
ACCUMULATIVE PERCENTAGE OF 50-CERCARIA SAMPLES HAVING MAXIMUM PENETRATION INDICATION
MAXIMUM NUMBER OF CERCARIAE PENETRATING PER 50-CERCARIAE SAMPLE

FIGURE 12. ACCUMULATIVE FREQUENCY DISTRIBUTION SHOWING PERCENTAGE 50-CERCARIAE SAMPLES IN WHICH THE MAXIMUM NUMBER PENETRATING WAS ≤ THAT INDICATED. DATA ARE BY CLASS INTERVALS OF 3

DISCUSSION

Evidence of the development of resistance to reinfection following an initial infection with schistosomes has been given by Ozawa (1930) in five dogs believed to have been cured of a first infection with *S. japonicum* by injections of sodium antimony tartrate; by Fairley, Mackie, and Jasudasan (1930) in monkeys with *S. spindale*, to which, however, they are naturally comparatively resistant; and by Vogel (1949) in monkeys with *S. japonicum* after a series of preliminary infections. In these studies, the total recovery of fewer or less well-developed parasites, the finding of fewer eggs in the feces, a reduced death rate, or less severe clinical symptoms in these hosts serially exposed than in control animals not previously infected were considered acceptable evidence of immunity. The primary infection in each case was permitted to mature before the challenging infection was given. This evidence seems to indicate that under the conditions and in the hosts described above, as well as in the young mice in this study, fewer of the schistosomes were able to mature in hosts which already had a mature infection than in those which did not.

A more accurate check on the number of schistosomes maturing from each exposure is available however. The parasites of the initial and challenging infections may be tagged by their sex. This technique, of course, checks only the effect of a single sex of worms on a superinfection, also unisexual and of the opposite sex. The fact that no immunity was observed at the one week and one month intervals, while the development of an immunity was noted at the two month interval between exposures, suggests the possibility that eggs and their contained miracidia may play a major part in the immunological response. On the other hand, the age of the mice, the comparative size of the worms and the length of time they have been in contact with host tissues may also be responsible factors.

The reduced parasite burdens from the challenging infections which followed the first exposures by one day or less were of the greatest interest. This observation suggested immediately the working hypothesis that recent penetration of the skin of the mice by homologous cercariae might inhibit penetration of subsequent cercariae. This appeared to be the case under the conditions of this experiment, though as has been shown, the effect was a reduction in numbers penetrating and was never absolute. The reduction in any group, however, showed statistical significance at the 0.1 per cent level between the values for penetration of the experimental and control mice at one hour, five hour, and one day intervals between exposures. The suppression of subsequent penetration varied in individual mice: in some it was very effective; in most, it was marked; in a few, the number of cercariae penetrating approximated that of the controls. This variation was not attributable to differences in the numbers of cercariae penetrating initially, within the narrow limits of variation recorded here. It may be that initial exposures of 500 cercariae per mouse, for instance, will reduce the challenging penetration more than did the present initial exposure of 50

cercariae per mouse. Work is in progress on this phase of the problem of inhibition of cercarial penetration.

The inhibiting reaction occurs quickly. Within less than two hours the number of penetrating cercariae of the challenging infection was reduced to a significant degree. This partial barrier to penetration was effective for less than two days. The difference between penetration in the experimental and control mice, when the interval between exposures was two days, was not of statistical significance even at the five per cent level, nor did it become so again for the longer intervals studied.

The absence of any inhibition of the challenging infection one week after the primary cercarial invasion is especially noteworthy in view of histopathological studies of mouse tail skin penetrated by cercariae now in progress in this laboratory. This work will be published in a later paper, but it may be noted here that schistosomulae, apparently living, growing and normal, were still present in small numbers just under the stratum corneum and in much larger numbers in the Malphigian layer, in the hair follicle along the hair and within the sebaceous glands, as well as in the dermis and within the venules as long as seven days after exposure. Yet at the interexposure interval of one week there was no difference at all in numbers of cercariae penetrating the tail skin of previously exposed mice and their controls. Obviously then, the phenomenon is not related to the mere presence of the cercariae in the skin, but to the recency of their penetration.

Inhibition of cercarial penetration at the challenging exposure appears to be a local reaction and to depend on previous recent cercarial penetration. Previous penetration through other skin areas had no perceptible influence on subsequent penetration through tail skin; and soaking the tail in water without cercariae could not be substituted for initial cercarial entry. It is not a reaction to handling, for the tail-soaking procedure required the same handling as did the tail exposure.

The question of cross-immunities with other parasites could not be considered in the study. Other parasites were present in many of the mice which were autopsied. Two nematodes were noted, *Syphacia obvelata* and *Nippostrongylus muris*; and *Hymenolepsis nana* was present in an occasional mouse. These parasites were found in about equal numbers in experimental and control mice as was expected, inasmuch as the mice were always taken at random from a cage of several recently weaned litters. Insofar as could be determined there was no relation between the presence of either the nematodes or the tapeworms and the number of schistosomes recovered.

References to an active inhibition of epidermal penetration by trematode larvae are few, and these are not closely applicable to the findings here. They should, however, be mentioned. A localized epidermal immunity to the ectoparasitic monogenetic trematode *Epibdella melleni* was acquired by the moonfish (*Vomer setipinnis*) after previous infection, but reinfection could occur through areas not penetrated earlier (Nigrelli and Breder 1934). Later Nigrelli (1937) showed that the mucus of certain immune fish, such as the dogfish, was lethal to these same parasites and that susceptible fish kept in tanks with elasmobranchs

did not become infected. He suggested that this mechanism thus protects these fish against the parasites.

A striking resistance to penetration of snails already harboring sporocysts and cercariae of *Cotylurus flabelliformis* was found in studies reviewed by Cort, Brackett, Olivier and Nolf (1945). Uninfected control snails permitted the penetration of large numbers of the parasite. It was further observed that cercariae coming in contact with the body surfaces of infected snails reacted in a manner different from that characteristic of cercariae contacting uninfected snails. The latter quickly began to penetrate and soon disappeared within the soft tissues of the snail. The former coming in contact with the snail surface moved along it with a looping movement and soon swam away as if they had encountered an abnormal host.

Penetration of the epidermis of snail and fish hosts, so far removed phylogenetically from mammals, can scarcely be compared with the invasion of mouse skin. Certainly the cercariae of *S. mansoni* which had not penetrated during exposure gave no evidence of being in the presence of a lethal factor such as the mucus of naturally immune or immunized fish. With our present general lack of understanding of the mechanism of cercarial invasion, it seems impossible to relate this inhibition of penetration into recently exposed mice to the broad principles of immunity. Nor is there any gross evidence in mouse skin of any hypersensitivity to reinfection with *S. mansoni* such as Watari (1936) described in the skin of rats superexposed to *S. japonicum* and which seemed to retard migration of these cercariae within the skin tissues.

By way of speculation, however, the investigations of several authors may be referred to in this connection. Iturbe and Gonzalez (1919) suggested that in rats, which are not now considered as favorable hosts for *S. mansoni*, cercariae of this schistosome species penetrated the skin by way of hair follicles. Similarly, Watari (1936) found that many cercariae of *S. japonicum* invaded the skin of the chicken, an abnormal host, along the follicles of the downy feathers. If this is the usual route of penetration of mouse tail skin, then it is possible that a hair follicle recently used by a penetrating cercaria is not an open route for subsequently invading schistosomes for some time. On the other hand, if this is not the pattern of penetration and if cercariae enter through the inert unbroken stratum corneum, as believed by Gordon and Griffiths (1951), then it must be postulated that cercarial penetration stimulates the production of some substance which renders the stratum corneum more impervious, is repellant to subsequently presented cercariae, or sets up antibodies effective at the skin surface against a penetration enzyme.

SUMMARY AND CONCLUSIONS

Female albino mice were re-exposed to cercariae of *Schistosoma mansoni* at intervals of one hour, five hours, one day, two days, one week, one month and two months after an original exposure, together with previously unexposed control mice. Exposures were to 50 cercariae per mouse by tail for one hour. For all except the longest interexposure interval the parasites of the initial and challeng-

ing infections were tagged by their sex, and the mice were initially exposed at the age of about six weeks. When the interval between exposures was two months, each exposure was to both male and female cercariae, and the initial infection was presented to the mice when they were one week old. In all studies autopsies were performed six weeks after the second exposure.

The following conclusions have been drawn:

1. The percentage of schistosomes maturing from the challenging exposure in the superinfected mice was significantly reduced below that in the control mice when the interexposure interval was one hour, five hours, one day and two months.

2. On the other hand, the number of adult schistosomes maturing in the superinfected mice from challenging exposures presented two days, one week and one month after the initial exposure was essentially equal to that from specific control exposures.

3. This reduction was the result of a local inhibition of penetration by the cercariae of the challenging exposure when the second exposure followed the first by an interval of one hour, five hours and one day. Each exposure was to cercariae of one sex only.

4. When the interexposure period was longer than one day, no significant inhibition of penetration was noted. No clue was found to the mechanism of reduction of the worm burden from the challenging exposure when it was presented two months after the original exposure.

Acknowledgment

The author gratefully acknowledges the technical assistance of Carlos Schultz, HMC, USN, and John R. Hackey of the Naval Medical Research Institute.

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THE HISTOPATHOLOGY OF EXPERIMENTAL SCHISTOSOMIASIS

II. Bisexual Infections with *S. mansoni*, *S. japonicum*, and *S. haematobium*[1]

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Department of Preventive Medicine, College of Medicine, New York University

INTRODUCTION

In a previous paper (Meleney *et al.*, 1952) we described the hepatic lesions found in albino mice harboring unisexual or early bisexual infections with *Schistosoma mansoni*, and compared these lesions with those found in mice harboring similar infections with *S. japonicum* and *S. haematobium*. The purpose of the present paper is to describe the lesions encountered in mice harboring bisexual infections of longer duration, that is, after the beginning of egg deposition, and to point out similarities and differences in the lesions found in different experimental hosts harboring the same species of worm, and in the same species of host harboring a different species of worm.

REVIEW OF LITERATURE

Fairley (1920) infected monkeys with *S. mansoni* and *S. haematobium* and studied the lesions produced up to 14 weeks after infection. He stated that the eggs of the two species produced an identical type of lesion and he described the structure of the typical pseudotubercle and its development as far as early fibrosis. He also described the early lesions in the liver, spleen and kidneys of monkeys dying of heavy infections before the deposition of eggs apparently caused by toxic products of the worms, and concluded that the combination of these with the egg lesions "constitute the underlying basis of the various syndromes manifested in the early stages of bilharziasis in man."

Brumpt and Chevallier (1931) studied the histopathology of *S. mansoni* and *S. haematobium* infections in mice and found them essentially similar, consisting of two types, granulomatous produced by eggs and toxic caused by worms. In three rats harboring *S. mansoni* infections of 14 months duration, they described fibrous bands connecting portal spaces near the border of the liver where worms were present in portal venules, and called attention to the similarity of this lobulation to that normally occurring in pig's liver.

Koppisch (1937) followed in great detail the development of lesions produced by *S. mansoni* in rabbits and albino rats. Unfortunately the two hosts which he employed are not favorable for the normal development, reproduction and long survival of *S. mansoni*. For this reason most of the pathology described by Koppisch was produced by the worms congregating and dying in the liver. Eggs

[1] Supported by a grant (E27) from the National Institutes of Health, Public Health Service.

were not found in large numbers and more often were merely empty shells rather than normal eggs containing miracidia. In rats, "pig liver" lobulation was associated with worms in that organ, and no eggs or pathological lesions were found in the intestines.

Krakower, Hoffman and Axtmayer (1940) studied the reactions of white rats on normal and vitamin-A deficient diets to heavy infections with *S. mansoni*. In this relatively unsuitable host most of the adult worms remain in the liver instead of migrating to the mesenteric veins. The authors found that on a normal diet most of the worms were destroyed within four months by thrombotic and acute periportal cellular reaction followed by fibrosis and ultimate disappearance of lesions. In rats on a vitamin-A deficient diet the reaction to the worms was much less marked and most of them survived the experimental period. Where death of worms occurred, the surrounding reaction was more fibroblastic and there was less tendency to final resolution of the lesions. The comparatively few eggs deposited in the liver also produced more reaction in rats on a normal diet than in those on a vitamin-A deficient diet.

Krakower, Hoffman and Axtmayer (1943) also described the course of lesions produced in guinea pigs harboring massive infections with *S. mansoni*. The authors were interested mainly in the basis for production of portal hypertension which led to "congestive splenomegaly" and to the development of large portocaval anastomotic veins by which adult worms left the portal-mesenteric veins and reached the right heart and the lungs. They described the egg lesions in the liver from the acute stage through the tubercle and fibrous stages until the egg shells and even the fibrous tissue disappeared. They also described the lesions produced by dead worms in the portal venules which similarly progressed through acute and fibrous stages to final absorption and re-establishment of portal circulation. Since the guinea pig is a relatively unfavorable host for *S. mansoni* these authors found that most of the worms had died and had been absorbed by about nine months after infection, leaving the tissues of the liver, intestine and lungs practically normal in appearance. For this reason the progressive development of increasing fibrosis due to long-continued egg deposition was not seen.

Faust and Meleney (1924) described the pathology produced by *S. japonicum* in experimental animals. The period of egg deposition was studied in dogs. They described the "schistosomiasis abscess" as an acute reaction to the egg, and its transition to a pseudotubercle. In the liver these lesions were discrete, but in the wall of the intestine, where many eggs were deposited together, they were often large and confluent. No lesions directly associated with adult worms were described, and only insignificant cellular infiltrations independent of egg lesions. No evidence of cirrhosis of the liver attributable to the schistosome infection was found up to 119 days, the duration of their oldest infection.

MATERIAL

The histological sections on which this study is based were prepared from albino mice, hamsters, albino rats, guinea pigs and rabbits infected in the laboratory with *Schistosoma mansoni*, *S. japonicum* or *S. haematobium*. Infection was

produced in most cases by immersion in water containing cercariae, but mice and rats were sometimes infected by intraperitoneal injection. Cercariae from several snails were used to assure the production of bisexual infections. In almost all cases a known number of cercariae was employed. This made it possible to obtain a rough correlation between the intensity and duration of the infection and the severity of the resulting lesions. Animals were killed with ether, except for a few guinea pigs which died of intercurrent infection, and a few rabbits which apparently died of schistosomiasis. Tissues were fixed in formalin, Zenker's or Bouin's solution, and paraffin sections were stained with hematoxylin and eosin. The portal and mesenteric veins were inspected to detect the presence of worms and in many cases after tissue blocks had been taken the liver and mesenteric vessels were perfused by the method of Yolles *et al.* (1947) in order to determine the sexes and stage of development of the worms. Usually the macroscopic appearance of the liver, spleen, intestines and lungs was noted.

Much more material was available from infections with *S. mansoni* than with *S. japonicum* and *S. haematobium* because of the difficulty of establishing the life cycles of the two latter species in the laboratory.[2] It is our purpose, therefore, first to describe the development of lesions produced by *S. mansoni* in mice, then to compare these lesions with those produced by the same parasite in hamsters, rats, guinea pigs and rabbits, and finally to compare these lesions with those produced by *S. japonicum* and *S. haematobium* in the same species of animal, so far as available material and the suitability of the mammalian hosts permit.

The liver provides the best tissue in which to observe the development of lesions associated with eggs, which appear to contribute most to the histopathology of these infections. The liver will therefore receive principal consideration in interpreting the development of lesions. The intestine is the second important organ in which eggs are deposited in experimental infections, but the "looseness" of the mucosal and submucosal tissue makes the effect of the presence of eggs less uniform. The urinary bladder, lungs and spleen will receive consideration only in so far as they contribute to the pathological picture.

HISTOPATHOLOGY OF THE LIVER

I. S. mansoni *infections*

A. *Mice.* The histological sections which were studied were taken from 31 mice, each having been infected with 50 to 200 cercariae and sacrificed from 5 to 32 weeks after infection.

[2] Most of our infections with *S. japonicum* and *S. haematobium* were produced by cercariae from infected snails kindly sent to us from the endemic areas of these infections. It is a pleasure to acknowledge the cooperation, often involving considerable time and effort, of the following: Drs. C. K. Chu and C. P. Mao, National Institute of Health, Nanking, China; Dr. C. L. Kao, Shanghai, China; Maj. Warren Christopher, M.S.C., U. S. A. Shanghai; Col. G. W. Hunter, M.S.C., U. S. A. 406 Medical General Laboratory, Tokyo, Japan; Capt. J. Rutzky, M.C., U. S. A., Kyoto, Japan; Dr. C. H. Barlow and Dr. M. Abdel Azim, Ministry of Health, Cairo, Egypt; and Capt. J. J. Sapero, M.C., Comdr. R. A. Phillips, M.C. and Lt. R. E. Kuntz, M.S.C., Naval Medical Research Unit No. 3, Cairo.

1. *Periportal and parenchymal cellular infiltrations.* We have described previously (Meleney *et al.*, 1952) accumulations of mononuclear and polymorphonuclear cells in the periportal spaces of the liver in mice harboring unisexual and early bisexual infections with *S. mansoni*, *S. japonicum* and *S. haematobium*. In mice harboring unisexual infections with *S. mansoni*, such accumulations frequently extended far out into the parenchyma. They usually contained many polymorphonuclear leucocytes including eosinophiles. They increased in frequency and extent in infections up to about 12 weeks in duration. In older infections they tended to revert to the mononuclear type of cell and to be limited to periportal areas. Similar infiltrations were present in the livers of mice harboring bisexual infections prior to the deposition of eggs. They tended to be more extensive than in unisexual infections. We have interpreted these tissue reactions as evidence of sensitivity to the foreign protein of the worms present in the mesenteric-portal blood vessels, corresponding to the systemic reactions of fever, urticaria and eosinophilia described in man in the early stages of schistosome infections.

After the deposition of eggs in the liver in bisexual infections, these periportal cellular infiltrations persist and contribute to the total histopathological picture. Lesions produced by eggs, although independent in origin, often occur in the same localities so that the entire histopathology might be attributed to the eggs unless the periportal infiltrations are recognized as independent lesions.

2. *Lesions produced by eggs.* Eggs delivered from female worms into the larger vessels of the mesenteric-portal system are carried by the blood stream into the liver. They usually lodge in terminal venules in the portal spaces. Some are apparently carried into blood capillaries within liver lobules and some may pass through larger anastomoses between portal and hepatic venules and ultimately lodge in the lungs. This possibility is indicated by the fact that glass beads of a diameter up to 180 micra, injected into the portal vein, have been recovered from the lungs (Prinzmetal *et al.*, 1948). Since the uterus of the adult female of *S. mansoni* usually contains only a single egg at one time, its eggs are apparently discharged singly into the blood stream and this accounts for the fact that they are usually found singly in the liver in early and light infections, unless the worm is in a branch of the portal vein within the liver and deposits numerous eggs in one location.

At the time that eggs are expelled from the uterus of the female worm the embryo within the egg is usually not yet mature. It has been estimated by Vogel (1942) that the life of the miracidium of *S. japonicum* within the egg after deposition is limited to about three weeks. In histological sections, it is impossible to determine whether the miracidium within a given egg is still viable, because its structure and nuclei appear to be intact even in older lesions which are in the fibrotic stage. Egg shells containing no cellular material are sometimes found in the terminal venules or capillaries. They are usually collapsed and stimulate little or no cellular reaction from the surrounding tissue.

The first reaction to the embryonated egg in a terminal vessel is the proliferation of endothelium about it, separating it from the lumen of the vessel. Then

small mononuclear and polymorphonuclear leucocytes including eosinophiles accumulate in the surrounding tissue (Fig. 1) and the relationship of the egg to the vessel may no longer be apparent. Soon epithelioid cells appear adjacent to the egg and extend out into the lesion, leaving a ring of leucocytes at the periphery. This is the characteristic pseudotubercle (Fig. 2).

The earliest appearance of eggs in mice infected with *S. mansoni* was in infections of five weeks duration. By seven weeks the lesions in some mice were in all of the stages described above and in addition some of them had areas of total disintegration of the tissue around the egg (Fig. 3), with polymorphonuclear and mononuclear cells at the periphery, while others had advanced to the stage of fibrosis (Fig. 4). Fibroblasts develop concentrically about the egg, sometimes first adjacent to it, sometimes at the periphery of the lesion. They replace the epithelioid cells and as the fibrosis becomes more advanced the cellular elements decrease until a circumscribed scar is formed (Fig. 5). The leucocytes at the periphery of the pseudotubercle may persist into the scar stage, but eventually they disappear. During the pseudotubercle stage foreign-body giant cells may appear (Fig. 6) and are also often present in the fibrous stage, usually adjacent to the egg, sometimes completely surrounding it or even invading it and replacing the miracidium.

The last recognizable stage of the egg lesion appeared only in mice harboring infections of 18 weeks or more in duration. In this stage the scar tissue was reduced in amount and was practically acellular, with the egg shell still visible, though sometimes only faintly discernible. This indicates that the egg shell is ultimately absorbed by the host. Calcification of eggs was not detected in the livers of any of our *S. mansoni* mice, the longest infection being 32 weeks in duration.

Since the eggs of *S. mansoni* are delivered singly, the lesions in the liver were usually discrete, containing only one egg. In heavy infections, however, two or more eggs seemed to have produced a single lesion, or the lesions were close enough together to become confluent. In these cases the connective tissue of the portal spaces also was often more abundant and denser, and sometimes contained eggs without typical lesions. In a few animals eggs were present in large groups, probably deposited by a female worm occupying a branch of the portal vein within the liver.

In heavy infections the egg lesions replaced large areas of parenchymal tissue, destroying the normal architecture of some lobules and producing scars which caused depression of the capsule and large deeper areas of scar tissue. There was little evidence, however, of general increase of fibrous tissue either between or within the liver lobules and the walls of the portal veins showed no general thickening independent of egg lesions. Thus the ultimate fibrosis of the liver in these *S. mansoni* mice appeared to be due entirely to the localized lesions produced by eggs. The periportal cellular infiltrations which were prominent before the deposition of eggs were less intense in infections older than 12 weeks and became largely mononuclear in type. There was no evidence that they led to the development of fibrosis.

3. *Lesions produced by adult worms.* Adult male and female worms were frequently encountered in liver sections. Often there was no apparent damage to the endothelium of the portal venule and no cellular infiltration in the periportal tissue. In other instances the vessel was partly or completely surrounded by wandering cells (Fig. 7), but these infiltrations could not be distinguished from those where worms were absent. In still other instances the worms were obviously dead and in various stages of disintegration, and the vessel and surrounding tissue showed changes accordingly. These consisted progressively of thrombosis of the vessel, loss of the endothelium with proliferation of subendothelial tissue invading the worm (Fig. 8), accumulation of mononuclear and polymorphonuclear cells in the periportal tissue and penetrating into the worm, and ultimately fibrosis of the area with scar formation, development of collateral venules and entire disintegration of the worm so that its presence could be recognized only by hematin pigment from its intestine.

4. *Other pathological findings.* Hematin pigment excreted from the intestines of adult worms became progressively more prominent in Kupffer cells and in histiocytes in the portal spaces and egg lesions, and it was often seen in large masses in the oldest infections, sometimes unassociated with cells, in the loose connective tissue of portal spaces or in dense scar tissue (Fig. 9). It did not appear to stimulate the proliferation of cells or the development of fibrosis.

Areas of "coagulative necrosis" of parenchymal cells were frequently present in infections up to 14 weeks in duration. They were similar to those previously described in unisexual and early bisexual infections (Jaffe *et al.*, 1945; Meleney *et al.*, 1952). In these areas the parenchymal cells stained bright red with eosin, and their nuclei were either shrunken and pyknotic or absent (Fig. 10).They were sharply demarcated from the surrounding normal parenchyma and some were invaded at the periphery by polymorphonuclear leucocytes. Their similarity to infarcts suggests that they may have been produced by the temporary obstruction of portal venules by schistosome worms, or by pressure occlusion of a branch of the hepatic artery. Rosenbaum and Egbert (1952) have described similar lesions in a human liver following ligation of the hepatic artery. They did not appear to be related to the development of fibrous scars, probably because of the ability of the parenchymal cells to regenerate.

B. *Hamsters.* The liver sections from hamsters harboring bisexual infections with *S. mansoni* were taken from 19 animals each exposed to 100–500 cercariae and sacrificed from 5½ to 16 weeks after exposure. The differences in histopathology from that encountered in mice may be summarized as follows.

1. *Periportal cellular infiltration.* This was generally less than in mice and was almost entirely mononuclear. It was present to a moderate degree in practically all animals.

2. *Lesions produced by eggs.* Eggs in portal venules without any surrounding cellular reaction, apparently recent arrivals, were present in all animals, in contrast to the mice, in which such eggs were rarely seen in infections longer than seven weeks in duration. There were fewer acute egg lesions containing polymorphonuclear leucocytes. The pseudotubercles by the seventh week were mostly

very "foamy" in appearance, composed of loose connective tissue with a peripheral ring of mononuclear leucocytes and fibroblasts (Fig. 11). By 14 weeks they had become smaller, and many had lost the peripheral ring of cells. Early egg lesions were also present in these older infections. Foreign-body giant cells were very rarely present in the lesions. There was no generalized fibrosis of the liver, which could be termed cirrhosis, independent of the egg lesions.

3. *Other findings.* Although adult worms were present in the liver sections of many hamsters, no damage to the adjacent vessel was seen and no dead worms with the accompanying reaction to their presence were encountered. A few small areas of red-staining "coagulative necrosis" of parenchymatous cells were encountered, but they were much less common than in mice.

C. *Rats.* Albino rats are unsatisfactory hosts for *S. mansoni.* In our previous observations (Moore *et al.*, 1949) only 0.9 per cent of the cercariae injected intraperitoneally and only 2.3 per cent of those employed for percutaneous infections were recovered as adult worms. This is in sharp contrast to our observations on mice and hamsters, in which the average proportion of cercariae recovered as adult worms was over 20 per cent. In rats the worms also developed more slowly than in mice and hamsters, were smaller and were almost always recovered only from the liver, very few having been able to migrate back to the mesenteric veins. Eggs were never deposited in the wall of the intestine.

The liver sections in the present study were taken from 19 rats harboring bisexual infections 6 to 19 weeks in duration and employing 100 to 15,000 cercariae per rat. Infection was produced by intraperitoneal injection in 15 rats and by cutaneous exposure in 4. The differences in histopathology from that produced in mice were as follows.

1. *Periportal cellular infiltration.* In only two rats was even a moderate accumulation of mononuclear and polymorphonuclear leucocytes seen in the portal spaces independent of the presence of eggs or dead worms.

2. *Lesions produced by eggs.* The eggs were usually collapsed or contained unorganized material more often than well-developed miracidia. They were rarely numerous and were usually near the capsule of the liver or close to worms which themselves had produced tissue reaction in the same area. No eggs were seen in some infections of 7 to 9 weeks duration in which 500 to 15,000 cercariae had been employed. A few of the egg lesions were in an early stage, containing neutrophile and eosinophile leucocytes, but most of them were in the pseudotubercle stage even though the eggs were collapsed. Most of the pseudotubercles were smaller than those in mice and hamsters. Some had gone on to partial fibrosis (Fig. 12). Extensive proliferation of fibrous tissue sharply outlining lobules was associated with lesions in several rats (Fig. 13). This is similar to the "pig liver" fibrosis described by Brumpt and Chevallier (1931) and by Koppisch (1937). It was limited mainly to the lobules near the capsule of the liver where most of the eggs were deposited. It was present in the absence of worms from the sections but it is possible that dead worms may have been present in nearby vessels and may have been responsible for the fibrosis.

3. *Lesions produced by worms.* Adult worms were seen in liver sections of 12

rats with infections from 6 to 14 weeks in duration. Some worms were normal in appearance, others were in various stages of disintegration. In some animals several pairs of worms were in large cystic blood spaces at the edges of lobes. Where the worms were normal in appearance, the tissue about the vessel sometimes showed no cellular reaction; in other cases there was accumulation of mononuclear and polymorphonuclear cells including eosinophiles. Where the worms were dead they were in various stages of disintegration, invaded by cells and fibrous tissue from the vessel wall with accumulation of leucocytes in the surrounding tissue and increase of fibrous tissue extending into and between lobules to form scars. The interlobular fibrosis decreased progressively from the affected vessel outward, other portions of the section showing normal architecture. Some portal venules associated with or near those containing dead worms were widely dilated and often showed polypoid projections into the lumen. In other cases there were many small venules in the surrounding connective tissue, apparently representing canalization of obstructed larger vessels (Fig. 14).

4. *Other pathological findings.* Hematin pigment deposited in macrophages and Kupffer cells was less in amount than in mice and hamsters, in spite of the large number of cercariae employed in some infections. No areas of "coagulative necrosis" were observed in any rats. This is interesting in view of the fact that most of the worms remained in the liver.

Thus the histopathology of *S. mansoni* infection in the rat is the reaction to a parasite which is unable to develop and complete its normal migration, or even to survive very long in that host.

D. *Guinea pigs.* These animals are also unsatisfactory experimental hosts for *S. mansoni*. In our paper on comparison of hosts (Moore *et al.*, 1949) it was reported that although from 5 to 60 per cent of cercariae employed for percutaneous infection were recovered as adult worms, they matured slowly and were smaller than those from mice and hamsters and eggs were never recovered from the feces.

Our histological sections were taken from 6 guinea pigs infected percutaneously, using 2,000–4,600 cercariae and sacrificing the animals from 6½ to 12 weeks after infection. The findings in the liver may be summarized as follows. Very few eggs were present and they were almost always empty shells with little or no cellular reaction around them. Worms were present in all of the livers, usually dead and disintegrating, with thrombosis of portal venules, marked perivascular infiltration of polymorphonuclear and mononuclear cells, invasion of worms by fibroblasts, and, in the later infections, marked fibrosis of periportal tissue and proliferation of bile ducts. Periportal cellular infiltration, independent of dead worms, was insignificant. Areas of red-staining "coagulative necrosis" of parenchymal liver cells were seen in two animals.

E. *Rabbits.* Our previous observations (Moore *et al.*, 1949) demonstrated that rabbits are also not very satisfactory experimental hosts for *S. mansoni*. Although an average of 12 per cent of the cercariae employed in percutaneous infections were recovered as adult worms, the worms were slow in developing and were small in size, and eggs were not passed in the feces. It was the inability of eggs deposited in capillaries in the wall of the intestine to escape into the lumen

which first stimulated our interest in the comparative histopathology of the infection.

The liver sections in our present study were taken from 9 rabbits each exposed percutaneously to 5,000 cercariae and sacrificed 7 to 16 weeks after exposure. In addition, one rabbit was studied which had been exposed percutaneously to an unknown number of cercariae by Dr. H. W. Stunkard three years prior to sacrifice.

The distinctive features of the histopathology of the liver were as follows. All animals except the one with the three-year infection showed a moderate degree of periportal infiltration of mononuclear and a few polymorphonuclear leucocytes. Eggs were not found in infections of 7 to $7\frac{1}{2}$ weeks duration but were present at 8 weeks and thereafter. They usually consisted of empty shells, either without any surrounding tissue reaction, or surrounded by a foreign-body giant cell or a few mononuclear cells or a little fibrous tissue. Rarely a fibrosed pseudotubercle was seen. Adult worms were found, a few apparently living and with no reaction about the portal venule, but more often they were undergoing disintegration, with thrombosis and obliteration of the venule and extensive necrosis of the surrounding tissue (Fig. 15). Such areas appeared macroscopically as yellow spots on the surface of the liver measuring up to 2 mm. in diameter. More advanced lesions associated with dead worms consisted of widely dilated portal venules surrounded by fibrous tissue, or venules with polypoid projections of endothelium into the lumen of the vessel. In these areas there was usually considerable thickening of interlobular connective tissue. Macroscopically these older lesions appeared as fibrous scars at the edge of a lobe extending toward its dome. In the rabbit having an infection of three years duration, although about 200 living worms, including both males and females, were recovered from the mesenteric-portal veins by perfusion at autopsy, there was remarkably little pathological reaction in the liver. There was practically no periportal cellular infiltration, only slight increase of fibrous tissue in portal spaces, a few collapsed egg shells in the portal connective tissue and a few recently arrived eggs containing undifferentiated embryonic cells in terminal portal venules. A small amount of hematin pigment was present in mononuclear phagocytes in portal spaces and in Kupffer cells. The liver parenchyma appeared normal. It is obvious that, in this animal, neither the adult worms, after living for three years in the portal-mesenteric veins, nor their eggs had produced any significant cumulative or progressive pathological effect on the liver.

Summary of liver pathology in S. mansoni *infections.* In mice and hamsters the principal pathology is produced by fertile eggs, which cause an acute or subacute tissue reaction leading to fibrosis and obstruction to portal venules. This pathology is progressive because the worms survive, mainly in the mesenteric veins, and continue deposition of fertile eggs. Cellular reaction to living worms is inconstant and decreases with duration of infection. Diffuse cirrhosis independent of egg lesions does not occur. In rats, guinea pigs and rabbits the worms develop more slowly, are smaller, and most of their eggs fail to develop miracidia. These eggs act as innocuous foreign bodies, elicit very little cellular response and

therefore do not produce significant pathology.[3] The adult worms die much earlier than in mice and hamsters and produce larger localized lesions in and about the terminal portal venules in which they lodge. These lesions lead to scarring with ultimate restitution of portal circulation. In the rat there is a tendency to extensive proliferation of interlobular fibrous tissue surrounding the lesions produced by dead worms. This is not seen in guinea pigs and rabbits. Hematin pigment is present in histiocytes and Kupffer cells in proportion to the number, size and digestive activity of worms and the duration of the infection. It may be present in large masses in late infections in which many fibrosed egg lesions are present. It does not seem to contribute to the development of fibrosis.

H. S. japonicum *infections*

The adult female of *S. japonicum* may contain several hundred eggs in its uterus at one time and is therefore presumed to produce many more eggs than *S. mansoni*. This is reflected in the fact that mice will die of much lighter infections with *S. japonicum* than with *S. mansoni*. The range of mammalian hosts which pass eggs in the feces is also much wider for *S. japonicum* than for *S. mansoni*. There are, however, differences in suitability of various laboratory animals as experimental hosts for *S. japonicum*. Bang and Hairston (1946) reported that in albino rats the mature worms remained small and most of them stayed in the liver and that in guinea pigs only a small proportion of the eggs deposited in capillaries progressed to the development of a mature miracidium. Our own experiments on the recovery of adult *S. japonicum* from experimental animals, as yet unpublished, tend to confirm those observations. Our observations also indicate that mice, hamsters and rabbits are favorable experimental hosts.

The eggs of *S. japonicum* are often deposited in the blood vessels in large groups rather than singly as by *S. mansoni*. In portal venules in the liver they are sometimes found held together by a transparent substance which apparently comes from the uterus of the worm. The effect of large groups of eggs containing miracidia is the production of extensive abscesses with necrotic centers. In the mucosa or submucosa of the intestine these are likely to rupture into the lumen, as shown by Faust and Meleney (1924) in dogs. In deeper tissues of the intestinal wall or in other organs such as the liver, these abscesses ultimately lead to larger scars than are usually encountered in *S. mansoni* infections.

Our general impression is that the individual egg of *S. japonicum* is more toxic to the tissues of the host than is that of *S. mansoni*. Not only is the cellular reaction usually more acute, but the egg tends to stain more intensely with eosin, indicating a more acid reaction, and there is often a flame-like, red-staining material extending outward from the egg shell, as depicted by Hoeppli (1932). Koppisch (1941) has also observed this "eosinophilic fringe" in human and mon-

[3] Our longest infection in a guinea pig was 12 weeks in duration. Krakower *et al.* (1943) described acute and fibroblastic lesions about eggs in the liver of massively infected guinea pigs, reaching a peak of intensity at about 21 weeks after infection. Longer infections in our guinea pigs might have shown similar lesions produced by fertilized eggs.

key infections with *S. mansoni*, but in our experience the fringe is more frequent and extensive about eggs of *S. japonicum* (Fig. 16).

Difficulty in obtaining infected *Oncomelania* in sufficient numbers to produce balanced bisexual infections limited the number of animals suitable for histopathological study, but our material is sufficient to permit a reasonable comparison with *S. mansoni* infections.

A. *Mice.* The 9 mice from which sections were studied had been infected from 4 to 25 weeks, using from 25 to 150 cercariae per mouse. Periportal cellular infiltration, which was only slight in unisexual infections, was intense in four of these mice. Eggs without surrounding cellular reaction were present in portal venules in the 4-weeks infection, and were also found in later infections. They were often adherent to each other in large groups lying loosely in the lumen of a large venule or caught at a bifurcation (Fig. 17). In infections of 6 to 10 weeks duration, egg lesions were present in all stages from acute abscesses with many eosinophile cells through the pseudotubercle stage with giant cells, to fibrosis. In two animals with 6-weeks infections there were peculiar areas in which the liver parenchyma was replaced by loose fibrous tissue distal to the deposition of groups of eggs in portal venules. In two animals necrosis with nuclear fragmentation was present at the center of egg lesions. In the 25-weeks infection polymorphonuclear leucocytes were still present in some lesions, but there were many masses of calcified eggs in dense or loose scar tissue (Fig. 18). Areas of red-staining "coagulative necrosis" were present in three animals, usually adjacent to egg lesions. A dead worm was encountered in only one animal, in which the portal venule containing the worm was thrombosed and surrounded by young fibrous tissue and polymorphonuclear leucocytes.

B. *Hamsters.* The 6 hamsters from which sections were studied had infections ranging from 4 to 24 weeks in duration using 325 to 375 cercariae per hamster. Some of the infections had few female worms, resulting in less egg deposition than in well-balanced infections. Periportal cellular infiltration was less intense than in mice. Eggs in portal venules without surrounding cellular reaction were found in a 4-weeks infection. At 5 weeks and later, egg lesions were present. Each lesion usually contained only one egg. They showed acute polymorphonuclear accumulations in the early stage, with later pseudotubercle and giant cell development leading to fibrosis. In one infection of 22 weeks duration there was extensive fibrous scarring, with calcification of eggs and very heavy deposition of hematin pigment extracellularly in the scar tissue (Fig. 9). Between the scars there was proliferation of parenchymal tissue without normal lobulation. No dead worms were found in the livers of hamsters.

C. *Rats.* The 5 rats from which liver sections were studied harbored infections from 4 to 10 weeks in duration, using 720 to 1000 cercariae per rat. Only slight periportal mononuclear cell infiltration occurred in any of these rats. Eggs were found in the liver of all the animals, at 4 weeks with only slight mononuclear cell reaction, at 5 weeks with acute polymorphonuclear reaction and later with tubercle formation and fibrosis. The egg lesions, however, were smaller than in mice and hamsters, although several eggs were often present in one lesion. Giant

cells were numerous in older lesions, and fibrous strands between lobules were prominent as in rats infected with *S. mansoni*. Only one disintegrating worm was found. It was in the 10-weeks infection and was imbedded in an organized thrombus on one side of a large portal venule. No areas of "coagulative necrosis" were seen. Hematin pigment was less prominent than in mice and hamsters.

D. *Rabbits.* The 12 rabbits from which liver sections were studied harbored infections ranging from 8 to 101 weeks in duration, using from 500 to 2,000 cercariae per rabbit. These rabbits were all infected for the purpose of maintaining a supply of eggs with which to infect snails. Most of them died of late complications of the infection, thus presenting the end results of continuous activity by the worms. The earliest stages of egg lesions were not seen. Only a brief statement of the later pathology can be given here.

The results of egg deposition by *S. japonicum* in the liver of rabbits are more dramatic than in the smaller animals considered above. In the rabbit dying 8 weeks after percutaneous exposure to 500 cercariae there were many egg lesions in which the egg was surrounded by a wide zone of necrotic cells showing nuclear fragmentation, this zone in turn surrounded by epithelioid cells. The difference from the lesions in smaller animals was a third peripheral zone of dense fibrous tissue sharply demarcated from surrounding normal parenchyma (Fig. 19). Some lesions had advanced entirely to the stage of fibrosis and there was great increase of fibrous tissue in portal spaces and extending between them, forming a definite cirrhosis out of all proportion to the number of eggs deposited. In older infections the fibrosis was greater, sometimes forming large scars which greatly distorted the contour of the liver. Eggs were scattered through the fibrous tissue singly and in groups, in all stages of degeneration including calcification. Hematin pigment was often present in extracellular masses. Compensatory proliferation of parenchyma was evident. In animals in which only a few female worms had developed there were much fewer eggs, but there was considerable interlobular fibrosis. Two rabbits were found at autopsy to have unisexual infections; one male, one female. In neither of these animals did the liver show any histopathology, indicating that the severe lesions in bisexual infections were caused mainly or entirely by eggs.

In contrast to *S. mansoni* infections in rabbits, no dead worms with the resulting histopathology were encountered in the liver sections of *S. japonicum* rabbits.

III. *S. haematobium infections*

Our material for study was limited, first by the difficulty in procuring infected *Bulinus* snails, and second by the fact that *S. haematobium* has a very narrow range of suitable mammalian hosts. Hamsters were the only hosts used by us in which eggs are passed regularly in the feces. Mice pass eggs less consistently. Our sections were taken only from mice and hamsters. Four rats and six guinea pigs were exposed percutaneously to *S. haematobium* cercariae from several snails, but at autopsy, 4 to 14 weeks after exposure, only a few male worms were recovered by perfusion from three of the rats and one guinea pig, and no evidence of egg deposition or of dead worms was seen in liver sections.

S. haematobium develops to maturity more slowly than *S. mansoni* or *S. japonicum*. The earliest infection in which eggs were found in the liver was in a hamster infection of nine weeks duration. The female worm may hold as many as 30 eggs in the uterus at one time, and the eggs are often deposited in the tissues in groups.

A. *Mice*. The 5 mice from which liver sections were studied harbored infections from 12 to 42 weeks in duration, using 100 to 150 cercariae per mouse. The histopathology was much less distinctive than in mice infected with *S. mansoni* or *S. japonicum*. In each animal there was moderate or intense periportal cellular infiltration, with polymorphonuclear leucocytes usually predominating. In the 12-weeks infection there was a large area of red-staining "coagulative necrosis" involving several liver lobules. The eggs were few, and deposited singly or in small groups, a few surrounded by pseudotubercles, but most were empty shells surrounded by giant cells or fibrous tissue. No worms were seen in any of the liver sections. A small amount of hematin pigment in phagocytic cells in some animals was evidence that worms had been present in the portal-mesenteric veins.

B. *Hamsters*. The 13 hamsters from which liver sections were studied were from infections 5 to 75 weeks in duration, using 200 to 500 cercariae per hamster. The first evidence of pathology coincided with the beginning of egg deposition in the 11-weeks infection. Many of the early eggs were empty shells, but a few contained miracidia and were surrounded by pseudotubercles. No acute abscesses were seen. Two weeks later "foamy" tubercles like those in hamsters with *S. mansoni* infections were seen. The next infection in our series was of 26 weeks duration. By that time extensive interlobular fibrosis and large scars had developed, with eggs singly and in masses in the scar tissue. In longer infections recently deposited eggs were sometimes found and occasional pseudotubercles, but the predominating picture was fibrosis outlining areas of normal parenchyma. Heavy deposits of hematin pigment were present in some animals. Adult worms were encountered frequently in portal venules, but no pathology resulting from dead worms was seen.

HISTOPATHOLOGY OF THE INTESTINE

I. *S. mansoni infections*

Eggs were first found in sections of intestine one-half to one week later than in the liver. They appeared first in capillaries of the mucosa and submucosa, later in the muscularis and serosa. In mice many of the eggs in the mucosa and submucosa were densely surrounded by eosinophile cells, but others had elicited little or no cellular reaction. Progression of lesions through the pseudotubercle stage to fibrosis was seen in the submucosa and even more in the muscularis and serosa, where thickening and nodular masses developed. Eggs were usually deposited singly but were also seen in single file in a capillary, or in a small group. In late infections they were occasionally in large groups. Rarely an egg was seen in the lumen of a mucosal gland or breaking through the epithelium with or without a few leucocytes.

In hamsters there was much less polymorphonuclear reaction in the early lesions. Pseudotubercles were the predominating type of early lesion and had the loose "foamy" structure seen in the liver. These lesions progressed to complete fibrosis.

In rats no eggs were found in any sections of intestine, and in guinea pigs they were rarely encountered and were almost all empty shells.

In rabbits eggs were found in sections of the intestine in infections of 8 weeks and longer. They were mostly in the mucosa and submucosa. Although some contained miracidia, there was usually no cellular reaction about them. In the rabbit with an infection of three years duration, eggs had never been detected in the feces. At autopsy about 200 male and female worms were recovered from the liver and mesenteric veins by perfusion. No macroscopic lesions were seen on the serosal surface of the intestine, but snips of tissue from all levels from duodenum to colon contained eggs. The entire contents of the cecum were washed and centrifuged, and one egg was found. Sections from the duodenum showed a few eggs, singly or in small groups in the mucosa and submucosa, a few containing miracidia, but most were collapsed and empty. There was little or no cellular reaction about them and no thickening or scar formation.

II. *S.* japonicum *infections*

As in the liver, the eggs of *S. japonicum* produced much more intense lesions in the intestine than did the eggs of *S. mansoni*. In addition to more acute lesions about single eggs, the deposition of eggs in large groups produced larger lesions in the form of abscesses, often necrotic at the center. These occurred mostly in the submucosa, muscularis and serosa, were uncommon in the mucosa, although many eggs were deposited there. All types of animals studied, namely mice, hamsters, rats and rabbits, had lesions of this type. In the mucosa many eggs were present, often spread out in large numbers in a group of capillaries tributary to a vein, and usually with very little or no cellular reaction. In the deeper layers of the intestinal wall the pseudotubercle stage of lesions was rarely seen. Instead the abscesses became fibrosed from the periphery inward, finally leaving a nodular scar containing many eggs, often calcified. The result of long continued egg deposition was seen best in rabbits. Nodular masses several centimeters in diameter protruded from the peritoneal surface of the small or large intestine, usually containing cheesy material in which were myriads of calcified eggs. Living worms were often found in the mesenteric venules adjacent to these masses. Worms were sometimes found also in anastomotic veins between the colon and the pelvis, and in two rabbits brown spots in the peritoneum just above the pelvis were found to contain masses of eggs. In one rabbit worms had reached the urinary bladder (see below).

III. *S.* haematobium *infections*

Although we have found eggs of *S. haematobium* in the feces of mice as early as 9 weeks after infection, the earliest mouse infection in which eggs were found in our intestinal sections was 14 weeks in duration. A few collapsed eggs were

present in the submucosa of the colon and the surrounding tissue was densely infiltrated with polymorphonuclear cells. In older infections, 32 to 35 weeks in duration, nodules were seen grossly on the ileum and colon and sections showed lesions in the submucosa, muscularis and serosa, in the pseudotubercle and fibrous stages. In the pseudotubercles some eggs contained miracidia and many polymorphonuclear leucocytes were present among the epithelioid cells. In hamsters the earliest infection which showed eggs in the intestine was 11 weeks in duration. There was no cellular reaction about these eggs. Our series contains no infections between 13 and 26 weeks in duration. In infections of 26 weeks and longer the colon and sometimes the ileum showed nodular masses on the peritoneal surface, and sections showed myriads of eggs, mostly in fibrous scars in the submucosa, muscularis and serosa, but also many eggs in the mucosa, usually without any cellular reaction and occasionally penetrating through the epithelium. Many eggs contained well-developed miracidia; some were calcified. No large areas of necrosis, forming abscesses as in rabbits infected with *S. japonicum*, were seen.

HISTOPATHOLOGY OF THE URINARY BLADDER

The only infections in this study in which the urinary bladder was involved were in hamsters infected with *S. haematobium* and in one rabbit infected with *S. japonicum*. In all six of the hamsters harboring *S. haematobium* infections from 33 to 67 weeks in duration, worms had crossed over from the portal system through the hemorrhoidal plexus to the systemic veins and had deposited eggs in the venules of the bladder. Worms were seen in these veins in some animals. In several of these hamsters granular thickening of the bladder wall was seen grossly. In sections eggs were found in the subepithelial tissue and between muscle cells (Fig. 20). Most of the lesions were in the fibrous stage. No ulceration or polyposis of the epithelium was seen in the sections.

The one rabbit infected with *S. japonicum* in which the worms had migrated to the bladder had been exposed to 2000 cercariae and died 14 weeks later. There were intense pathological lesions of the liver, intestines and lungs. The bladder had a large nodular mass on the peritoneal surface and eggs were found in the bloody urine. Sections showed many acute abscesses containing eggs and surrounded by fibrosis (Fig. 21) and several male and female worms in adjacent veins (Fig. 22).

HISTOPATHOLOGY OF THE LUNGS

In the post-cercarial stage the schistosome worms pass through the lungs in their migration from the skin to the mesenteric-portal veins but do not produce permanent pathology. Although we have occasionally found immature worms in the lungs of mice and rabbits infected with *S. mansoni*, we are convinced that most, if not all, schistosomes which develop to maturity in man do so primarily in the portal-mesenteric veins. Both adult worms and eggs, however, are found in the lungs at a later period in the infection and there has been much speculation as to the route or routes by which they reach there. The two possibilities are

(1) from the liver through anastomoses between the portal and hepatic veins, and (2) from the mesenteric veins through anastomoses with systemic veins. The route through the liver seems possible for eggs because of the observation of Prinzmetal *et al.* (1948) that glass beads of a diameter up to 180 micra, injected into the portal vein of rabbits, could be recovered from the lungs. This would not so easily account for the passage of adult worms to the lungs, but it is conceivable that they could work their way from portal venules through the parenchyma or through anastomoses into hepatic venules. The evidence is much better, however, for the passage of both eggs and worms through anastomoses from mesenteric to systemic veins. It is obvious that *S. haematobium* preferably crosses over from the lower mesenteric to the pelvic systemic veins through the hemorrhoidal plexus. *S. mansoni* also does this sometimes in man, and *S. japonicum* did it in one of our rabbits. However, the frequency with which eggs and adult worms were found in the lungs in our experimental animals suggests that other anastomoses may be involved. Krakower, Hoffman and Axtmayer (1943) described enlarged anastomotic veins between the upper mesenteric veins and the vena cava or its branches in guinea pigs infected with *S. mansoni*, in which massive egg deposition and death of worms in the liver had caused portal obstruction. Worms were seen in these anastomotic veins and many were recovered from the lungs. It seems probable that eggs as well as worms may pass through such anastomoses in other types of experimental animals as well as in man. It is well known that pulmonary fibrosis and *cor pulmonale* sometimes occur in man as late complications of *S. mansoni* infection (Clark and Graef, 1935), and extensive involvement of the lungs with egg abscesses was described in one early case of *S. japonicum* infection in an American soldier in the Philippines (Weinberg and Tillinghast, 1946).

In our experimental animals the lungs were involved as follows. In *S. mansoni* infections (Moore *et al.*, 1949) mice first showed eggs in press preparations in 8-weeks infections. Worms and eggs were first found in lung sections at 9 weeks, and frequently in longer infections. In hamsters there was similar lung involvement. In one rat exposed to 15,000 cercariae and sacrificed 9 weeks later, eggs were found in the lungs in press preparations, and 589 worms were recovered from the lungs by perfusion. In rabbits eggs and worms were occasionally found in the lungs in press preparations and sections.

Our series of animals infected with *S. japonicum* is too small to make accurate comparison with *S. mansoni*. However, eggs and worms were found in the lungs of *S. japonicum* mice, hamsters, rats and rabbits. Among our six mice infected with *S. haematobium*, eggs were found in one, in press preparations of the lungs. Among our 13 hamsters from which sections were taken eggs were found in most of the lungs in infections of 11 weeks and longer, and worms were seen in two infections of 26 and 53 weeks in duration.

The histopathology associated with eggs in the lungs was comparable to that in the other organs of the same animals. It was most dramatic in *S. japonicum* infections, especially in rabbits. In these animals early infections (8 to 10 weeks) showed acute egg abscesses with localized pneumonitis and often central necrosis

and giant cell formation. Longer infections showed in addition massive areas of scarring with calcified eggs and proliferation of bronchial epithelium into adjacent alveoli. Two of these rabbits had considerable fluid in the pleural cavities and fibrinous pleural adhesions.

Adult worms in the lungs were either in terminal arteries or rarely in dilated alveolar cavities. Some worms had produced no tissue reaction; others were in process of disintegration, with thrombosis of the vessel and intense perivascular accumulation of leucocytes. In longer infections these lesions had gone on to scar formation.

HISTOPATHOLOGY OF THE SPLEEN

It is beyond the scope of this paper to deal with the problem of splenomegaly in schistosome infections. In general the spleen was enlarged in proportion to the intensity and duration of the infection, but was not consistently so. In some infections of long duration there was considerable fibrosis of the splenic pulp. In some mice with relatively early infections with *S. mansoni* areas of necrosis were present in the pulp (Fig. 23). In the very prolonged *S. japonicum* infections in rabbits the spleen capsule was greatly thickened and there was a great increase of fibrous tissue throughout the organ, with diminution in size of the lymphoid follicles (Fig. 24). Eggs were occasionally found in the spleen, deposited by worms which had migrated into the branches of the splenic vein. The lesions produced by eggs were similar to those in the liver.

SUMMARY AND CONCLUSIONS

1. The histopathological lesions produced in bisexual infections with *Schistosoma mansoni* in experimentally infected mice, hamsters, rats, guinea pigs and rabbits have been described, and have been compared with those produced by *S. japonicum* and *S. haematobium*.

2. In hosts in which the worms mature with greatest rapidity and attain the largest size, fertile eggs are deposited in, or are carried to, the smallest blood vessels. The most typical lesions are those seen in the liver. They are circumscribed, are more or less acute at first, develop into a characteristic pseudotubercle and finally result in a scar. The intensity of the pathology depends upon the number of eggs deposited.

3. In hosts in which the worms mature slowly and remain relatively small, egg deposition is delayed and most of the eggs may be unfertilized and appear as collapsed shells, producing little or no acute reaction or ultimate scarring of the tissue.

4. The eggs of *S. japonicum* usually produce more severe lesions than those of *S. mansoni* or *S. haematobium*, partly because they are often deposited in large groups, and partly because each fertilized egg seems to exert a more acute toxic effect on the surrounding tissue.

5. Living worms in terminal vessels do not apparently stimulate a tissue reaction, but dead worms stimulate thrombosis of the vessel and an intense perivascular reaction which leads to disappearance of the worm, scar formation and

often recanalization of the vessel. Dead worms are found more frequently in unsuitable hosts.

6. In the liver periportal infiltration of leucocytes, described previously in unisexual infections, is also found in bisexual infections. It is more acute and extensive in early infections than later and is more frequent and intense in *S. mansoni* than in *S. japonicum* and *S. haematobium* infections. It does not appear to contribute significantly to ultimate fibrosis of the liver and is interpreted as an allergic reaction to the worms inhabiting the portal-mesenteric veins.

7. Areas of red-staining "coagulative necrosis" of liver parenchyma, previously described in unisexual infections, are also sometimes found in bisexual infections regardless of the duration of the infection. They may represent infarcts caused by occlusion of a portal venule or hepatic arteriole.

8. In the animals studied there was no visible evidence that the mere presence of living worms in the portal-mesenteric veins contributed significantly to the development of cirrhosis of the liver.

9. In the mucosa and submucosa of the intestine fertile eggs often do not stimulate much tissue reaction, but in the muscularis and serosa the egg lesions are similar to those in the liver.

10. In the urinary bladder lesions produced by eggs of *S. japonicum* and *S. haematobium* are similar to those in the intestine.

11. Eggs and adult worms of all three species were found in the lungs in some animals of each species employed in these experiments. This was probably the result mainly of migration of worms from the portal-mesenteric veins through anastomoses into the pelvic systemic veins or into the vena cava in the upper abdomen. Enlargement of these anastomoses, caused by obstruction to the portal circulation in the liver, probably facilitates passage of both worms and eggs through the anastomoses.

12. Lesions produced in the lungs by eggs were similar to those produced in the liver by the same species of egg. Worms were found mostly in arterioles but occasionally in alveolar cavities. Living worms caused little or no tissue reaction, but dead worms produced thrombosis of vessels and intense perivascular cellular reaction and ultimate scarring. In some late infections with *S. japonicum* in rabbits the lung pathology was intense, with fibrinous pleural adhesions and fluid in the pleural cavities.

13. In the spleen, areas of focal necrosis were found in some of the mice with early *S. mansoni* infections, and increase of fibrous tissue was seen in some late infections. Extensive fibrosis and thickening of the capsule were present in some late infections with *S. japonicum*. Egg lesions were sometimes found in the spleen.

14. From the histological observations in this study it is concluded that most of the pathology in the species of animals employed is produced by fertilized eggs and dead worms, forming localized lesions which, if numerous enough, lead to extensive scarring. If the infection becomes inactive or is terminated, and if the scars are not too extensive they may entirely disappear, and normal structure and function of the tissues may be restored. The early periportal cellular infiltration in the liver is interpreted as an allergic phenomenon resulting from the

presence of worms in the portal-mesenteric veins. It decreases as the infection continues, and does not appear to contribute significantly to ultimate cirrhosis of the liver.

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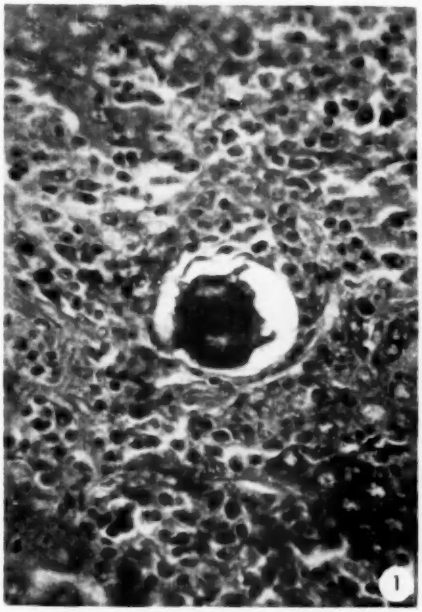
PLATE I

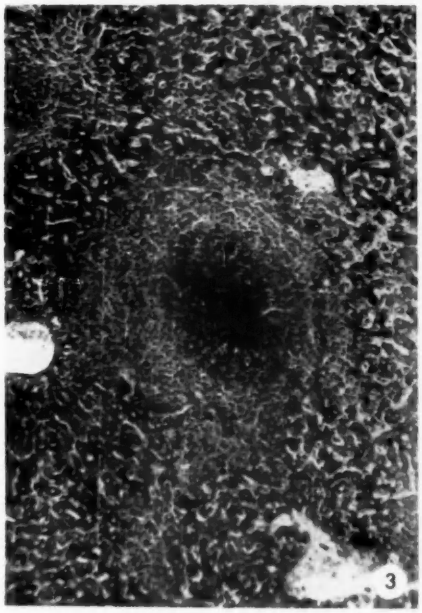
FIG. 1. *S. mansoni*, mouse, 7 weeks infection, liver. Egg in portal venule with beginning periportal cellular infiltration. ×400

FIG. 2. *S. mansoni*, mouse 7, weeks infection, liver. Egg lesion, pseudotubercle with cellular infiltration at periphery. ×120

FIG. 3. *S. mansoni*, mouse, 7 weeks infection, liver. Egg lesion, pseudotubercle with central necrosis. Egg not in section. ×100

FIG. 4. *S. mansoni*, mouse, 7 weeks infection, liver. Egg lesion, pseudotubercle with beginning fibrosis adjacent to egg. ×100





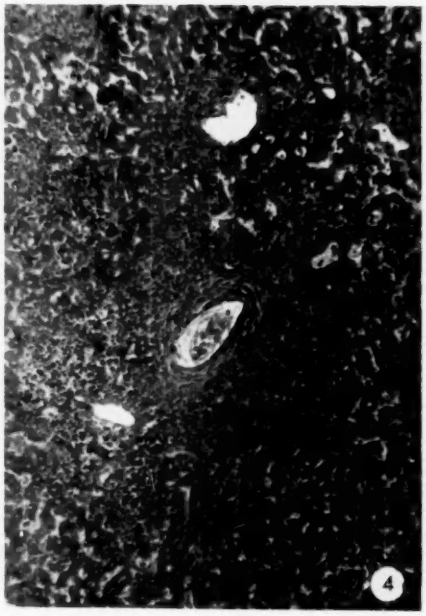


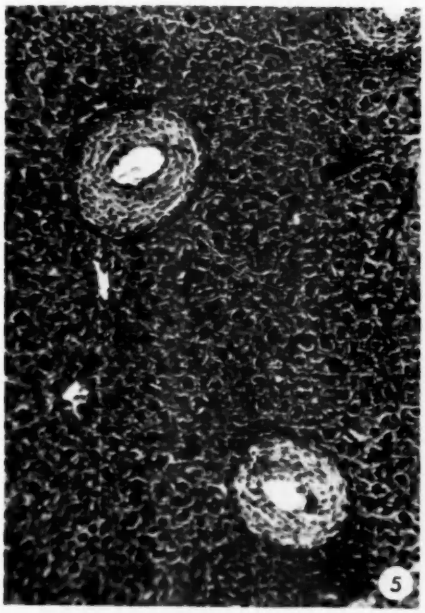
PLATE II

FIG. 5. *S. mansoni*, mouse, 26 weeks infection, liver. Egg lesions completely fibrosed with slight cellular infiltration at periphery. ×100

FIG. 6. *S. mansoni*, mouse, 11 weeks infection, liver. Egg partly surrounded by giant cell. ×500

FIG. 7. *S. mansoni*, mouse, 7 weeks infection, liver. Cross section of worm in portal venule with periportal cellular infiltration. ×100

FIG. 8. *S. mansoni*, mouse, 11 weeks infection, liver. Disintegrated worm in portal venule invaded by leucocytes and fibrous tissue, with extensive periportal cellular infiltration. ×100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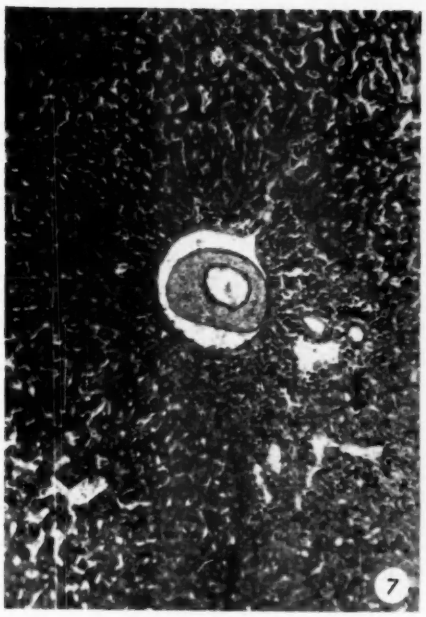
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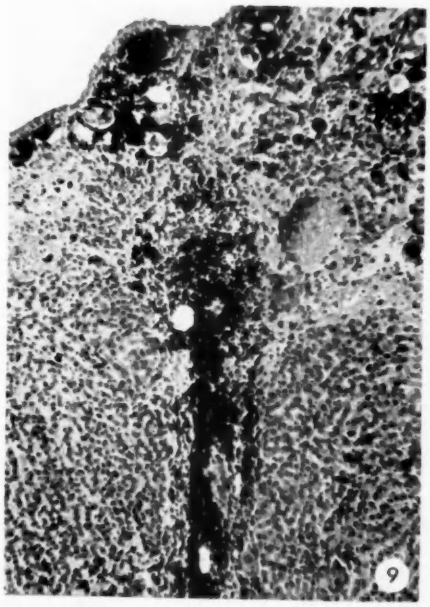
Plate III

Fig. 9. *S. japonicum* hamster, 22 weeks infection, liver. Large masses of extracellular haematin pigment in scars of egg lesions, and small masses in Kupffer cells. ×80

Fig. 10. *S. mansoni*, mouse, 7 weeks infection, liver. Red staining area of "coagulative necrosis" in parenchyma, surrounded by leucocytes. ×120

Fig. 11. *S. mansoni*, hamster, 7 weeks infection, liver. "Foamy" egg lesions consisting of loose fibrous tissue. ×100

Fig. 12. *S. mansoni*, rat, 7 weeks infection, liver. Small egg lesions, pseudotubercle stage, partly fibrosed. ×100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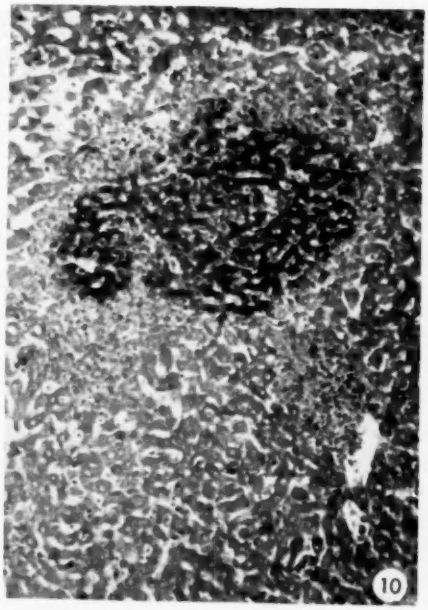
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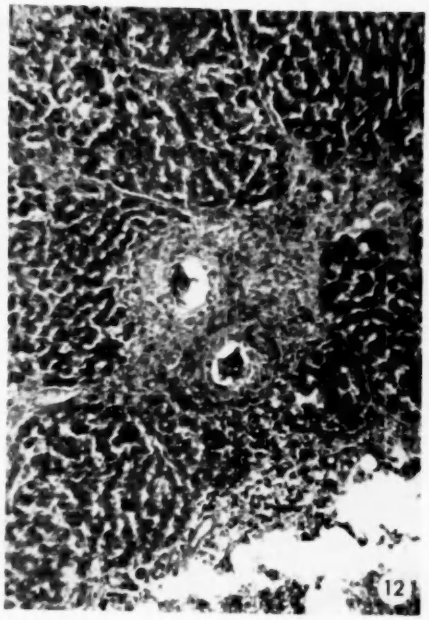
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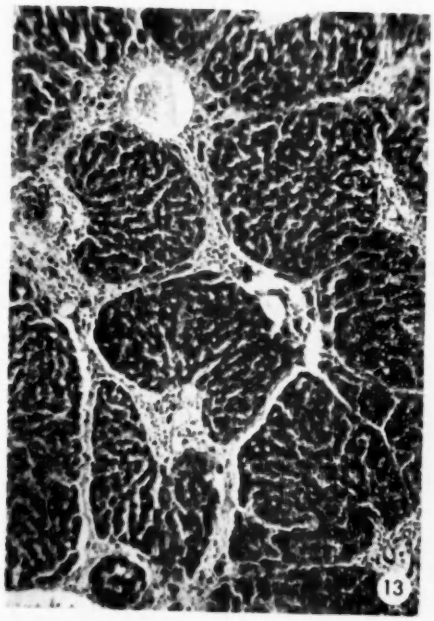
PLATE IV

FIG. 13. *S. mansoni*, rat, 10 weeks infection, liver. Interlobular fibrosis adjacent to portal venule obstructed by dead worm. ×80

FIG. 14. *S. mansoni*, rat, 7 weeks infection, liver. Section of female worm (bottom) with fibrosis and canalization of portal venule obstructed by dead worm. ×80

FIG. 15. *S. mansoni*, rabbit, 10 weeks infection, liver. Section of dead worm in large necrotic area just beneath capsule. ×50

FIG. 16. *S. japonicum*, rabbit, 14 weeks infection, lung. Red-staining "flame" reaction about egg, surrounded by polymorphonuclear leucocytes. ×320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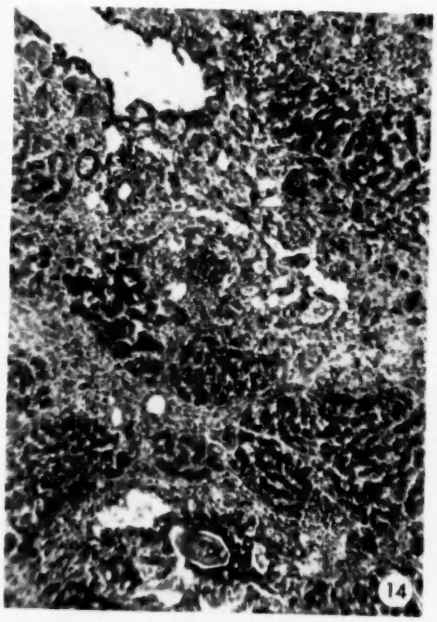
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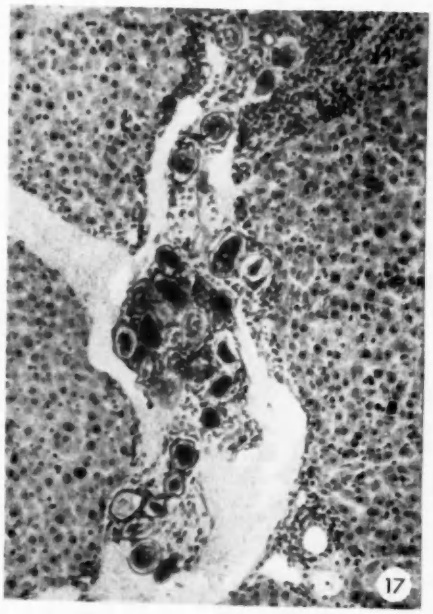
PLATE V

FIG. 17. *S. japonicum*, mouse, 6 weeks infection, liver. Mass of eggs forming an embolus in portal venule, with endothelial proliferation about eggs and beginning leucocytic infiltration about venule. ×100

FIG. 18. *S. japonicum*, mouse, 25 weeks infection, liver. Mass of calcified eggs in scar tissue with leucocytic infiltration persisting at periphery. ×100

FIG. 19. *S. japonicum*, rabbit, 8 weeks infection, liver. Lesion produced by mass of eggs, showing red staining necrosis at center, leucocytic infiltration at periphery of necrotic area and large ring of scar tissue. ×80

FIG. 20. *S. haematobium*, hamster, 33 weeks infection, urinary bladder. Eggs in submucosa. ×80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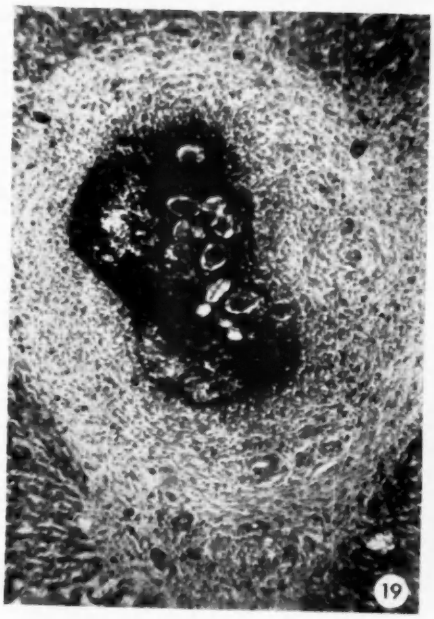
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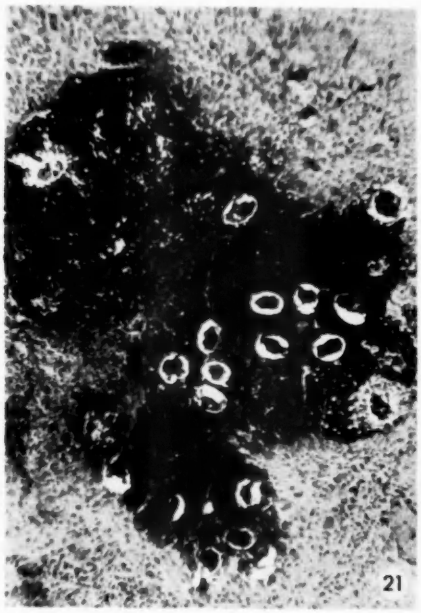
PLATE VI

FIG. 21. *S. japonicum*, rabbit, 14 weeks infection, urinary bladder. Lesion produced in bladder wall by mass of eggs, showing red-staining necrotic area surrounded by leucocytes and fibrous tissue. ×80

FIG. 22. *S. japonicum*, rabbit, 14 weeks infection, urinary bladder. Sections of female worm in vein showing eggs in uterus. ×100

FIG. 23. *S. mansoni*, mouse, $7\frac{1}{2}$ weeks infection, spleen. Necrotic area in splenic pulp. ×80

FIG. 24. *S. japonicum*, rabbit, 36 weeks infection, spleen. Thickened capsule, fibrosis of splenic pulp, wide blood sinuses, and deposits of haematin pigment. ×80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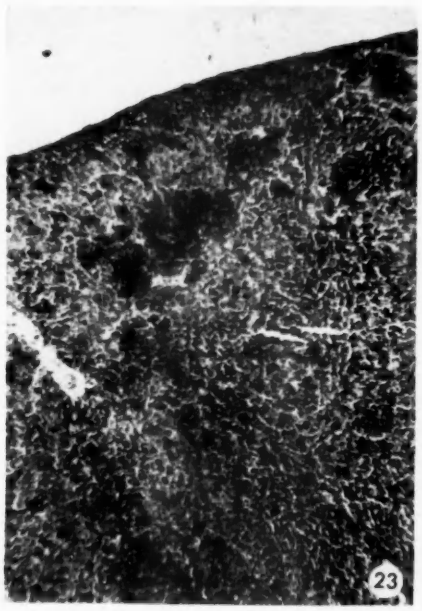
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THE DISTRIBUTION OF THE SNAIL *ONCOMELANIA NOSOPHORA*, INTERMEDIATE HOST OF *SCHISTOSOMA JAPONICUM*, ALONG THE TONE RIVER, JAPAN[1]

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The actual status of schistosomiasis japonica and the distribution of the intermediate host have never been adequately determined for the environs of the Tone River, which crosses the broad Kanto Plain of Central Honshu, Japan. Wright *et al.* stated (1947) that the entire sector of the river valley from Sakai to Sawara (Fig. 1) should be considered a suspected endemic area, with foci at Sakura, Sawara and possibly Fukawa. Olivier (1948) likewise referred to this section as the one least understood of the five endemic centers in Japan. He reviewed early studies made along the Tone, where the disease was first recognized *per se* by Miyagawa and Mizobuchi (1914). Takagi (1914, 1915) and Kobayashi and Takagi (1915) located snails at most points searched on the north side of the river between the Kinu and Kogai tributaries (Fig. 1), while on the south side of this same sector they were found only between Fukuda and Abiko. Except for the above section of the Tone, intensive surveys of the snail host distribution have not been made. Wright *et al.* (1947) looked for *Oncomelania nosophora* in the Sawara area without success, while Olivier (1948) relocated it in the vicinity of Koya.

In the fall of 1948 a search for *O. nosophora* was initiated in conjunction with our epidemiologic survey for intestinal parasites in Chiba, Ibaraki and Saitama prefectures (Ritchie *et al.* 1950, in preparation). During May 1950 this epidemiologic survey, with emphasis on the distribution of the snail, was extended to include the entire section of the Tone River from Sakai to Sawara, and in the following year a 15 mile sector was added below Sawara, a total distance of about 75 miles. Isolated endemic foci of schistosomiasis at Hikonari and Towa villages (Saitama) and Sakura (Chiba) were surveyed as well as parts of the Edo River, an auxiliary of the Tone (Fig. 1).

METHODS

Points of search were largely, though not entirely, made in the marsh areas between dikes or natural embankments and the river channel. This practice was followed because initial searches indicated that *O. nosophora* was essentially limited to such areas. With some exceptions, places of search were selected at

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approximately one-half mile intervals. On the basis of certain favorable environmental conditions which had been recognized early in the investigation, exact points of search were more critically selected by scanning the area from the top of dikes. A team of four or five individuals would then scatter out, making a through examination of the favorable site and converging if snails were found at that particular spot. At least 15 to 30 minutes were spent in checking each place selected; Japanese school children and adults sometimes assisted.

At the isolated foci of Hikonari and Towa, the Naka River and its lateral drainage ditches as well as irrigation ditches were repeatedly and thoroughly examined at the focal points of the disease. Consideration was given to the possibility of cercariae being carried some distance downstream, as was noted on

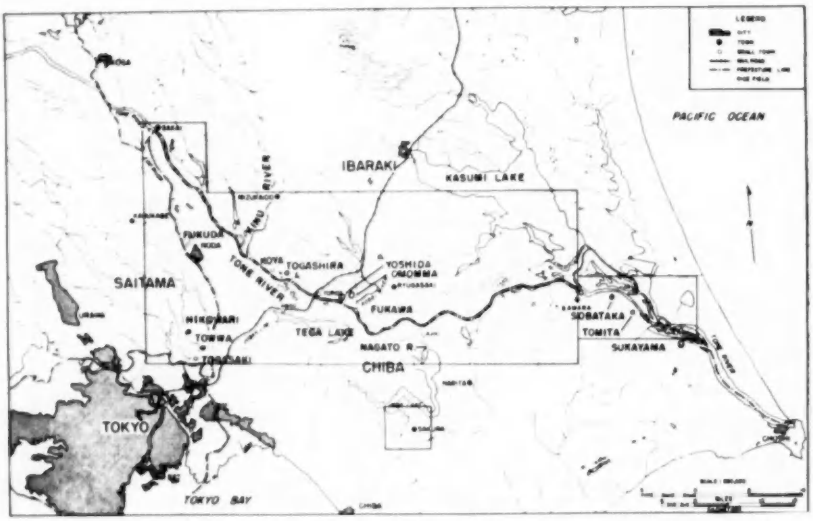

FIG. 1. Orientation map of Tone River Valley, showing areas surveyed for *Oncomelania nosophora*.

Leyte, P. I. (1946). At Sakura the previously recognized population center of *O. nosophora* was examined.

For convenience of reference, the surveyed portion of the Tone is arbitrarily divided into sectors as follows:

1. Sakai and above (5 miles). (Hikonari and Towa are located about 25 miles south of this point.)
2. From Sakai to the Kinu tributary (15 miles).
3. Between the Kinu and Kogai tributaries (10 miles).
4. Between the Kogai and Nagato tributaries (8 miles). (Sakura is located about 10 miles south of the Nagato junction.)
5. From the Nagato junction to Sawara (20 miles).
6. From Sawara to Sukayama (15 miles).

TABLE 1

Occurrence of Oncomelania nosophora *along the Tone River (1948–1951)*

South side of river	(From Sakai downriver)	*North side of river*	
LOCALITY	NO. SNAILS COLLECTED	LOCALITY	NO. SNAILS COLLECTED
	At Sakai		
Goka-Mura	107	Sakai-Machi	‡
	From Sakai to Kinu River Junction		
Sekiyado-Machi	0	Morito-Mura	‡
Kawama-Mura	0	Nagasu-Mura (0%)	0
Asahi-Mura	8	Nanago-Mura (0%)	0
Fukuda-Mura (0%)	0		
	From Kinu River to Kogai River Junction		
Tanaka-Mura (12%)		Ono-Mura (2%)	407
Hananoi	767	Koya-Mura (10%)	
Tomisei-Mura (0%)		* Koya (a)	107
Kujiya	1	* Koya (b)	848
Abiko-Machi (11%)		Inadoi-Mura (10%)	
Shibasaki	22	* Togashira	81
Aoyama (a)	7	Nonoi	17
Aoyama (b)	2	Ina	27
† Kohoku-Mura (1%)	0	Toride-Machi (5%)	
Fusa-Machi	0	Toride (a)	81
		Toride (b)	366
		Yoshida	16
		Omomma-Mura (5%)	
		Minami	1
		Todai	32
	From Kogai to Nagato River Junction		
Omori-Machi	0	Fukawa-Machi	‡
Fukama-Mura	0	Manaita-Mura (9%)	
Manaita-Mura		Daitoku (a)	13
Idetsu	2	Daitoku (b)	11
	From Nagato River to Sawara		
Ajiki-Machi	0	Genseida-Mura	‡
Toyozumi-Mura		Nagasao-Mura	‡
Tatsudai	15	Kanaezu-Mura	0
Takaoka-Mura		Toyoshima-Mura	
Takaoka	1	Yotsuya (a)	0
* Kobuke	15	Yotsuya (b)	17
Kozaki-Machi		Oshisuna	0
Kozaki	10	Rokkaku	4
Ima	0	Motoshinjima-Machi	
Higashiōta-Mura		Sawara (a)	162
Kawajiri	226	Sawara (b)	38
Tatajima	6		

TABLE 1—*Continued*

LOCALITY	NO. SNAILS COLLECTED	LOCALITY	NO. SNAILS COLLECTED
From Sawara to Sakayama			
Sawara-Machi		Tsunomiya-Mura	
Shinowara	101	Tsunomiya (a)	4
Okura-Mura		Tsunomiya (b)	150
Sobataka	23	Toyoura	
Okura	30	Tomita	35
Toyoura-Mura			
Tomita	22		
Sasakawa-Machi			
Sukayama	130		
Isolated foci			
Hikonari Mura (2%)			
(Saitama)	0		
* Towa Mura (14%)			
(Saitama)	43		
Sakura (0%)			
(Chiba)	0		

* Infected snails found.

† Aged shells found outside levee at Kobori in the bank of a small lake.

‡ Environment unsuitable, not searched.

Percentages placed after localities indicate incidence of human schistosomiasis.

FINDINGS

Summary of snail findings and occurrence of infections of Schistosoma japonicum. *Oncomelania nosophora* was found at forty distinct localities in the Tone Valley (Table 1). Twenty of these were on the north side of the river, nineteen on the south and one in Towa village (Saitama). Ten of the forty collections included over 100 specimens. Their locations undoubtedly represented established colonies, which was supported by the presence of young snails in some instances. Snails were located as far upriver as Sakai, and the most densely populated sectors appeared to be located (a) between the levels of the Kinu and Kogai tributaries, and (b) below the city of Sawara, well downriver.

Of the forty collections, infections of *S. japonicum* occurred in five. Three of these were in the Kinu-Kogai sector (Koya and Togashira), one was in the Nagato-Sawara sector (Kobuke) and the fifth was in Towa village.

Snail findings in relation to conditions of habitat. The environment of *O. nosophora* in the marsh areas within the levees of the Tone appeared to differ, at least superficially, from certain other endemic centers where snails are limited largely to irrigation ditches. To be sure, certain basic requirements of moisture and shade prevailed. Distinctive features of the Tone environment which contributed to a favorable habitat included:

1. Considerable width between levees and channel.

2. Occurrence of recesses, sharply defined coves or merely curves in dikes and natural embankments serving as protection against flood-water currents.
3. Soil containing considerable amounts of organic materials.
4. Heavy vegetation, such as marsh grass or reeds, scrub trees and brush-clumps of willow and/or briar.
5. Dampness of soil resulting from dew, rain or subterranean water, and maintained along with shade by denseness of vegetation.

These conditions were combined in varying degrees for the several river sectors listed above. (See Figs. 2, 3, 4.)

FIG. 2. View from north dike of Tone above Sawara roadbridge; both coarse reeds and finer vegetation were dense and snails were found at "A" & "B".

For a distance of five miles above Sakai, conditions were obviously unfavorable for snails. At the level of Sakai, however, there was an area of heavy vegetation on the south side immediately above the water-gate of the auxiliary Edo River (which was originally the primary river below this point); a sizeable, well established colony was located here.

Between Sakai and the Kinu River junction conditions were relatively unfavorable and only a single collection of eight snails was made. The soil was very sandy and vegetation was sparse, or when seemingly abundant, was thinly distributed over the gound.

Between the Kinu and Kogai tributaries all features of the environment were favorable (Figure 4) and *O. nosophora* was frequently encountered, particularly

FIG. 3. View from natural embankment on north side of Tone at Todai, Omonma-mura; foreground is set in a deep cove and snails were located at "x" under scrub trees.

FIG. 4. Typical of many parts of the marsh areas between levees and channel, particularly in the Kinu-Kogai sector; snails were found often in the fine marsh grass and brush clumps.

on the north side where collections were made at most of the places searched, including four where more than 100 specimens were obtained (Table 1). At Ono village many specimens were found in November 1948 near the water-gate in a deep recess. Even in hibernation the snails were situated under a very wet mat of decomposing reeds. In 1950 this population was almost wiped out by a heavy layer of sand deposited by flood. Takagi (1915) reported snails in the vicinity of Ono. Two large collections about 400 yards apart were made at Koya. One of these was found by Olivier in 1946 (1948) and has been under observation for four years. Immediately below the rail bridge at Toride many snails have been collected near the levee over a period of three years. At Yoshida and Ommoma, specimens were found under thickets of scrubby trees located in deep recesses.

South of the Tone between the Kinu and Kogai tributaries, *O. nosophora* was found in considerable numbers over a relatively large area at Tanaka village, just below the Kinu junction. This collection was made in the fall and many snails were hibernating in small brush-clumps. The area was covered by fine marsh grass. Other collections were made as far downriver as the rail bridge at Aoyama. From this point to the level of the Kogai, specimens were not found, although the environment appeared to be favorable.

The Fukawa area, immediately below the Kogai River junction, was reported by Wright *et al.* (1947) as being a possible endemic focus of schistosomiasis. However, conditions of environment between the levees and channel were unfavorable for *O. nosophora* as the river bed was narrow, soils very sandy and vegetation sparse. Snails were not again found until the level of the Nagato River (eight miles below the Kogai) was reached.

From the Nagato to Sawara the marshy areas were of only moderate width, first on one side and then the other, while coves and recesses were infrequent. Small collections were made at a number of places, but a sizeable colony was not located until the vicinity of Sawara (at Kawajiri) was reached. Also, just above the road-bridge at Sawara, numerous specimens were encountered in dense coarse reeds.

Continuing downriver, numerous snails were found in coarse reeds on the large island just below Sawara. For a distance of about two miles below this, the river channel ran close to the dike, but subsequently the width between them increased and the environment again became more favorable. Snails were found at three of five points examined over a distance of four miles from Sobataka to Tomita. Further downriver, the area between levee and channel became inaccessible except by boat, by means of which a search was made at Sukayama. Much of the marsh area here was covered with water, in spite of the fact that rainfall had been light for some time. Although such a condition is not favorable for *O. nosophora* numerous specimens were found midway between dike and channel on high land.

On the north side of the river below Sawara conditions were unfavorable for a distance of about two miles, but beyond this, two collections were made in the vicinity of Tsunomiya. A third examination at Tomita was also successful. These three places by no means exhausted the favorable habitats.

EPIDEMIOLOGICAL OBSERVATIONS OF THREE ISOLATED FOCI

1. *Hikonari.* In 1948 Hirano (1948) reported 15 cases of schistosomiasis at Hikonari among a group of farmers who had hurriedly harvested a potato crop as rising waters covered their riverside fields within the levees of the Naka River. The specific place, as well as points immediately upstream including lateral drainage ditches, were thoroughly checked on several occasions but *O. nasophora* was not found.

2. *Towa.* The Naka was similarly examined in 1949 and 1950 near a focal point of disease at Towa village, where an incidence of 14 per cent was noted in 1948 (MS). Irrigation and roadside ditches of the area were also thoroughly searched, particular attention being paid to the home site of an infected four year old boy. The main irrigation ditch of this area was traced for about one mile to a canal, which in turn was also checked. In June, 1951, the search was repeated and on this occasion snails were finally discovered inside the dike of the Naka among dense reeds, just above a swimming site. Three of 43 specimens were infected.

3. *Sakura.* This village lies about 10 miles south of the Tone, with Lake Imba intervening (Fig. 1) and is a recognized center of schistosomiasis. Kashiwado *et al.* (1926) reported a serious outbreak in this vicinity in 1925 among inhabitants who had gone into marshy areas to catch fish coming out of Lake Imba with flood waters. About 100 cases were recognized, a few of which were fatal. The above authors did not find snails, but Fukutani (1925) after repeated searches finally discovered *O. nasophora* in Nego-mura and Uchigo-mura. At that time the density of the snail population was such that a single collector could obtain as many as 200 specimens in an hour. In the identical locality in 1950, twelve persons searching for a period of two hours failed to find any snails. In the interim between 1925 and 1950 levees were constructed, making possible a transformation of marshes into rice paddies. In 1945 one case of schistosomiasis was found among 165 school children in the area (1947). Our parasitological survey (MS) which included the same place did not reveal any cases; thus it appears that the threat of schistosomiasis has been greatly reduced in this former endemic focus at Sakura.

DISCUSSION

It is interesting to note that our findings for the river sector between the Kinu and Kogai tributaries are very similar to those of Takagi (1914, 1915) and Kobayashi and Takagi (1915) which were reported 35 years ago. This is striking in view of frequent floods which inundate snail habitats by as much as 15 to 20 feet. In one instance a colony has persisted for four years in spite of several very severe floods (Koya), whereas at Ono a large colony was essentially destroyed by heavy deposits of sand.

The current survey has revealed, apparently for the first time, that *O. nasophora* exists in considerable numbers all the way from the level of the Nagato tributary (Chiba) to Sukayama, a distance of about 35 miles. Snails were particularly common over the lower half of this sector, beginning a short distance above Sawara.

The snail colony at Sakai (Goka-mura) is of special interest, since, in addition to being the one farthest upriver, it is possibly linked geographically with the origin of colonies in Saitama Prefecture. If this inference is correct, the origin of the Saitama colonies must have preceeded construction of dikes along the Tone and/or Edo Rivers, as the Naka River where snails are now known to exist in Saitama has no direct connection with either of them. Rather, it drains lowlands outside the levees of the Tone and Edo.

Whether snails exist farther upriver than Sakai is conjectural, but observations have been made at a number of points for an additional distance of 40 to 50 miles and in all cases the conditions of the riverbed were unfavorable for snails, due to excess sand and sparse vegetation.

Snails were not found outside the main levees and this suggests that the earlier investigators were correct in their observations that snails are essentially limited to areas between the levees and river channels. Takagi (1914) and Mizobuchi (1914) believed infections were contracted when people went inside the dikes to cut marsh grass and reeds. Kobayashi and Takagi (1915) noted among school children that infected individuals lived in communities immediately adjacent to the river. Olivier (1948) making intense searches for snails at Koya, was able to find many snails just inside the levee, while immediately outside only two could be found. To prove this point conclusively, exhaustive and time-consuming examinations would be necessary. Probably the simplest approach would be to skin-test people at a number of levels along the river, beginning with communities adjacent to the levees and surveying progressively away from the river, both snail-free and snail-populated river sectors being included.

Since *O. nosophora* successfully adapted itself to conditions of tillage in other endemic areas of Japan, it is surprising to find snails lacking in the tilled areas just outside the Tone levees, while inside they thrive. That such a line of demarcation existed prior to levee construction is unlikely. Just what conditions led to the disappearance of snails when land reclamation was made possible by building dikes is not clear. It may be noted, however, that irrigation ditches are unnecessary and consequently lacking in such areas, whereas they constitute the chief snail habitat in other endemic centers where snails have become adapted to tilled areas. Also, levees hinder the escape of inundating waters which accumulate behind them, particularly during winter months when pumping stations and water gates are not kept in operation. It is possible that inundations, particularly during hibernation, are destructive to such snail populations. This was inferred by McMullen *et al.* (1951), but he was unable to accumulate evidence.

The marsh areas serving as the habitat of *O. nosophora* along the Tone may more nearly represent the natural habitat than the irrigation ditches and other conditions associated with farming. In the Kofu valley, the Katayama district of Hiroshima Prefecture and parts of the Kyushu endemic center, the snail has adapted well to conditions of tillage which may constitute a secondary adaptation. In the Numazu area of Shizuoka Prefecture the snails have not been very successful under tillage.

Interrogation of those living near the river indicated that exposures commonly

occur when people go into rising flood waters, particularly when catching fish emerging from the channel. It was also reported that dermatitis occurred only during the early stages of the flood, before the advent of the high muddy waters. It may thus be plausibly inferred that cercariae do not commonly account for infections at any great distance downriver from their point of origin, due to the dilution factor and the fact that people avoid high waters.

To achieve actual eradication of *O. nosophora* in the Tone area will be much more difficult than in most other endemic centers of Japan where snails are largely limited to terminal irrigation ditches and damp embankments of terraces. In the Tone marshes the snails are widely and often thinly scattered, though not uninterruptedly, over hundreds of acres. In such a situation it may be possible to apply the mollusicide successfully by plane or helicopter. As previously noted by Olivier (1948), the control of more populous colonies should greatly reduce the danger of human exposures. Fortunately, with a few exceptions, snail infections are infrequent and human incidence and parasite density are relatively low along the Tone (MS).

SUMMARY

An extensive survey of *Oncomelania nosophora*, the intermediate snail host of *Schistosoma japonicum* was made in the Tone River valley from Sakai to Sukayama, a distance of about 75 miles.

Snail collections were made at 40 different places, being about equally divided between the two sides of the river. Ten collections consisted of 100 or more specimens and infections of *S. japonicum* occurred in five. Various epidemiologic factors in the distribution of the snails are discussed.

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LABORATORY INFECTION OF THE AGOUTI, *DASYPROCTA AGUTI*, WITH *SCHISTOSOMA MANSONI*

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A variety of laboratory animals can be infected with *Schistosoma mansoni*. The infection in monkeys, hamsters and mice is not unlike that in man, while in guinea pigs, laboratory rats, cotton rats, gerbils, rabbits and cats infection can occur, but either its establishment is difficult or few eggs are passed in the feces (Moore *et al.*, 1949; Abdel Azim and Cowper, 1950; Stirewalt *et al.*, 1951).

The agouti, *Dasyprocta aguti*, a rodent of the guinea-pig family, occurs naturally in Mexico, Central America and South America, and is readily maintained in the laboratory. This rodent is about the size of a rabbit and, if it were found to be a satisfactory laboratory host of *S. mansoni*, it could be utilized in chemotherapeutic and pathological studies when a larger animal would be desirable. This investigation was initiated to determine the susceptibility of the agouti to *S. mansoni*.

MATERIALS AND METHODS

Host Animals. Six adult agouties were obtained for the study.

Cercariae. Infected *Australorbis glabratus* were placed in individual sputum bottles with 10 cc. of water and exposed to direct light from 10:00 A.M. to 3:00 P.M. to stimulate maximum emergence of cercariae. Each snail was removed from its container, the number of cercariae was estimated under a dissecting microscope and all water containing the infective larvae was pooled.

Infection of Animals. Five of the six agouties were exposed by bringing a shaved area of the back measuring 5 by 12.5 cm. in contact with the suspension of cercariae for 45 minutes. A special animal board was designed to permit this exposure with minimal danger to technical personnel (Price, in preparation). The animals were allowed to dry before they were returned to their cages. After exposure the water was examined for cercariae. The sixth agouti was not exposed and served as a control.

Determination of infection. Daily fecal examinations were begun one month after exposure. Both direct smear and sodium sulfate-acid-ether concentration (Hunter *et al.*, 1948) technics were performed on each sample of feces. Egg counts were made using the sedimentation residue from one gram of feces.

RESULTS

Microscopic examination of the water remaining after exposure of each agouti revealed only an occasional cercaria. During the subsequent four months no evidence of infection was noted in the experimental animals other than the presence of eggs in the feces, which were first recovered after an interval of

65–72 days (Table 1). During the remaining two months period of observation the number of eggs evacuated was sparse and there were periods of several days during which none could be demonstrated.

TABLE 1

S. mansoni infection and egg passage in the agouti

AGOUTI NO.	EXPOSURE, NO. CERCARIAE	FIRST EGG PASSAGE, DAYS AFTER EXPOSURE	MAXIMUM EGG COUNT	
			Per gram feces	Days after exposure
1	700	66	28	105
2	800	65	12	96
3	1200	72	10	69
4	900	69	5	69
5	900	68	9	93
6	Control	—	—	—

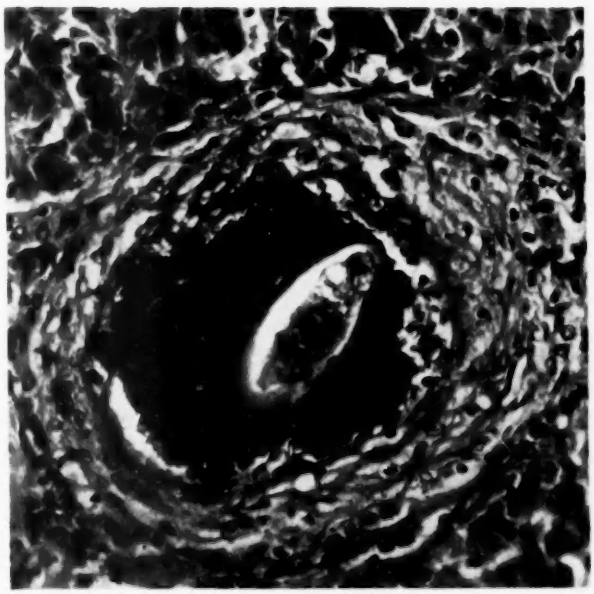

FIG. 1. Pseudotubercle with egg of *S. mansoni* in center, surrounded by dark zone of broken-down eosinophils and peripheral capsule of fibroblasts. 360 ×

The surviving agouties were sacrificed two months after the first eggs were encountered in the feces. Autopsy revealed an enlarged spleen and hobnail irregularities over the surface of the liver. Some of these nodules were aspirated and microscopic examination of the aspirate disclosed both viable and calcified *S. mansoni* eggs. Adult schistosomes were located in the portal and the larger

mesenteric veins; only a few worms were seen in the mesenteric venules close to the walls of the intestine. The mesentery of one agouti was completely dissected and from it 31 male and 27 female adult schistosomes, many in copula, were recovered. Histological sections were made of liver tissue. These were very kindly examined by Dr. Enrique Koppisch of the University of Puerto Rico, who reported that "practically all the portal fields presented one to three pseudotubercles, in many of which a schistosome ovum or shell was visible in the center. The pseudotubercles were composed of epithelioid cells mainly. Many of them were surrounded by a hyalinized fibrous capsule. There was no fibrosis of the portal spaces. They differed from those seen in man in that the ovum was frequently surrounded by a very prominent zone of strongly acidophilic material. This zone presented numerous pyknotic nuclei and probably represented broken down eosinophiles. A few worms were present in portal veins. About the necrotic portion of one of these worms, epithelioid cells had developed and had replaced the wall of the vein. A small amount of finely divided brown pigment was visible within some of the Kupffer cells" (Koppisch, personal communication). See Figures 1 and 2.

FIG. 2. Two worms of *S. mansoni* in intrahepatic portal vein. The epithelioid cell proliferation and eosinophilic infiltration is more marked about the disintegrating worm, left center than about the undamaged worm, right center. A calcified egg is also present, top center. 80 ×.

SUMMARY AND CONCLUSIONS

All of the agouties exposed to the cercariae of *S. mansoni* became infected, with eggs demonstrable in the feces 65–72 days after exposure. The majority of worms

were located in intrahepatic portal veins. Adult worms were also found in the mesenteric veins but very few were close to the intestinal wall, possibly accounting for the scant, irregular passage of eggs in the feces. The agouti, which occurs in many areas of tropical America where *S. mansoni* is endemic, supports the sexual cycle of this parasite and therefore might enter into the epidemiology of this disease. The following conclusions may be drawn:

1. The agouti is suceptible to schistosomiasis *mansoni*.

2. The agouti is suitable for laboratory studies with *S. mansoni* which do not require that eggs be passed in the feces.

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THE INFECTION OF *ANOPHELES QUADRIMACULATUS*, A HUMAN MALARIA VECTOR, WITH *PLASMODIUM CATHEMERIUM*, AN AVIAN MALARIA PARASITE

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At various intervals during the past six years, we have attempted to infect *Anopheles quadrimaculatus* with a number of strains of avian malaria including *Plasmodium elongatum*, *P. relictum* and *P. cathemerium* freshly isolated from English sparrows and used in both the duck and the canary. (Micks *et al.*, 1948; Micks, 1949a, 1949b) Without exception, these attempts were unsuccessful. Except for the infection of *A. quadrimaculatus* with exotic plasmodia such as *P. lophurae* (Coggeshall, 1940, 1941; Hurlbut and Hewitt, 1941, 1942; Jeffery, 1944) and *P. gallinaceum* (Hass and Akins, 1947; Cantrell and Jordan, 1949), there has not been until recently, convincing evidence that anopheline mosquitoes might be susceptible to indigenous avian malaria. It was not until 1946 (Sabrosky, McDaniel and Reider) when *A. crucians* in South Carolina was found to have a relatively high sporozoite rate, that anophelines were implicated in the epidemiology of avian malaria. More recently (1950) Frohne *et al.* reported on the persistence of infected *A. quadrimaculatus* and *A. crucians* in an area in the same state where human malaria parasitemia was low. These findings led Hunninen *et al.* (1950) and Hunninen (1951, 1953) to conduct a series of experiments which conclusively demonstrated that certain anopheline mosquitoes are capable of becoming infected with *P. relictum* in the English sparrow. The present report adds new information to our knowledge of the interrelationship of *Anopheles* mosquitoes and bird malaria.

MATERIALS AND METHODS

The mosquito species used were *Anopheles quadrimaculatus*[1] and *Culex quinquefasciatus*, both of which have been maintained as laboratory colonies for the past four years. The 3H-2 strain of *P. cathemerium*[2], maintained in the canary by continuous blood passage, was used in all experiments. The birds were immobilized by placing them dorsal side down on top of a cloth covered lantern chimney, parting the breast feathers and covering them with a piece of cheese cloth held in place by a rubber band. The canaries were then exposed to mosquitoes in a foot-square wire cage and the engorged ones were removed to a lantern chimney where they were maintained until dissection. The mosquitoes were kept at 25°C. and 80 per cent relative humidity from 8 to 21 days prior to dissection. When mature oocysts were present, the salivary glands were checked for sporozoites.

[1] Eggs with which to start this colony were kindly supplied by Dr. Richard Fay, Technical Development Branch, CDC., USPHS, Savannah, Georgia.

[2] Obtained through the kindness of Dr. Edith Box and Dr. Wendell Gingrich, University of Texas Medical Branch, Galveston, Texas.

RESULTS AND DISCUSSION

The results of experiments to determine the susceptibility of *A. quadrimaculatus* to *P. cathemerium* are shown in Table 1. In a series of ten experiments which involved a total of 120 *A. quadrimaculatus*, 31 or 25.8 per cent of the mosquitoes were infected. The number of oocysts per stomach varied from 3 to 600, averaging about 33, and they appeared normal in every respect. Sporozoites have not yet been found in the salivary glands of *A. quadrimaculatus* mosquitoes kept as long as 21 days after the infectious blood meal.

In two experiments, *C. quinquefasciatus* were fed simultaneously with the *Anopheles* and exhibited infection rates of 75 per cent and 93 per cent as compared with a 27 per cent and 50 per cent infection respectively in *A. quadrimaculatus*. It may be noted however, that the susceptibility differential in the two experiments is practically the same (48 and 43 per cent respectively).

TABLE 1

The susceptibility of Anopheles quadrimaculatus *to* Plasmodium cathemerium *in the canary (Compared with* Culex quinquefasciatus *in two simultaneous feedings)*

CANARY NUMBER	ANOPHELES QUADRIMACULATUS				CULEX QUINQUEFASCIATUS			
	Number dissected	Number infected	Average No. oocysts/ stomach	Per cent infection	Number dissected	Number infected	Average No. oocysts/ stomach	Per cent infection
C-105	13	1	20	8				
C-109	7	1	3	14				
C-113	14	2	16	14				
C-117	10	3	42	30				
C-119	29	8	73	28				
C-123	5	1	20	20				
C-126	11	3	50	27	20	15	184	75
C-126	9	3	11	33				
C-125	12	4	2	33				
C-134	10	5	95	50	14	13	155	93

Although Lucena (1938) has reported that Pessoa found one oocyst of *P. cathemerium* in *Anopheles strodei*, this observation has apparently never been repeated and therefore, has added little information as to the susceptibility of anophelines to avian malaria.

The present findings add a second species of native avian malaria to which *Anopheles* mosquitoes may be susceptible. It is interesting to note that while approximately 26 per cent of the *A. quadrimaculatus* used in our experiments were infected with *P. cathemerium* in the canary, Hunninen *et al.* (1950) found only one of 53 *A. quadrimaculatus* infected after feeding on the same host (canary) with *P. relictum* infection. However, when the English sparrow was used as the host for the latter parasite, Hunninen (1951) was able to infect 35 per cent of the *A. quadrimaculatus*.

Since we have only recently been able to infect *A. quadrimaculatus* with *P. cathemerium*, although both strains have been maintained in this laboratory for years, it may be that some genetic change has occurred in either the strain

of malaria, the mosquito or both. The fact that we have been always able to infect 75 to 85 per cent of our *C. quinquefasciatus* with this strain of *P. cathemerium* suggests an alteration in the mosquito (*Anopheles*) rather than in the strain of malaria.

SUMMARY

In a series of ten experiments which involved a total of 120 *A. quadrimaculatus*, 31 or 25.8 per cent were infected with *P. cathemerium* in the canary. The number of oocysts per stomach varied from 3 to 600, averaging 33. Although the oocysts matured and appeared normal in every respect, sporozoites have not yet been found in the salivary glands of *A. quadrimaculatus* kept as long as 21 days after the infectious blood meal. Two lots of *C. quinquefasciatus* fed simultaneously with the *Anopheles* revealed infection rates of 75 and 93 per cent.

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BREEDING MEDIA OF COMMON FLIES

I. In Urban Areas

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Field investigations of the effects of community-wide fly control upon the prevalence of diarrheal disease (Watt and Lindsay, 1948; Lindsay, Stewart and Watt, in press) revealed that the house fly, *Musca domestica* Linn., presented the principal problem in the control of flies with chemical insecticides. Recognition that the insecticides currently available were unsatisfactory in maintaining control of house flies in areas of high breeding potentials indicated the need for a return to basic procedures in preventing fly breeding. In a study of the effects of good municipal practice in refuse collection and disposal (Lindsay and McBrayer, 1950), it was found that prevention of fly production from garbage was inadequate to reduce fly prevalence in two study towns in south Georgia. Sanitational practices in the removal of animal pen and animal shelter refuse were found to permit fly breeding at a high level, and it was noted that the most abundant species were prolific breeders in such environments.

A review of the literature dealing with the muscoid flies revealed that wide ranges in larval habits as well as striking differences in adult breeding habits have been reported even among species of the same family. Few references were obtained in respect to specific breeding media and their potential application to numbers of larval or adult muscoid flies. The generalized way in which this particular phase of biological requirements of the muscoid flies has been recorded indicates the need of more specific information in regard to the various breeding media and the flies produced therefrom.

PLAN OF STUDY

The study, consisting of a preliminary phase and final phase, was conducted from early 1949 through April, 1951 in two towns, each with a population of approximately 15,000, located in southwest Georgia. The towns were some 30 miles apart and could be called typical southern agricultural centers. The mild climate permitted a long growing season for such items as tobacco, peanuts, cotton and truck crops. Animal exclusion ordinances did not exist or were not enforced in the two study towns and cows, pigs, chickens, ducks and rabbits were kept under insanitary conditions by many householders. Both towns were equipped with modern sewage disposal and water purification plants which serviced the bulk of the towns' areas. Each had a few industries; those of importance in connection with this study were meat-packing plants, peanut mills and livestock auction barns. Garbage pick-up was scheduled twice weekly within the limits of each town and the schedules were generally maintained.

The preliminary phase during the year 1949 established the kinds and occurrence of breeding media producing the more common muscoid flies. More than

30 naturally occurring media were found. Similar species composition from certain closely related items permitted the combination of these related items so that only twenty items of breeding material were used in the study. Five main categories, each consisting of items of similar origin, were established for further grouping of the breeding materials and to assist in the tabular presentation of data.

From these preliminary studies, a statistical design for a routine sampling schedule of all naturally occurring media was determined and the final phase of the study was established in May, 1950. The sampling design allowed for a comparison of the relative seasonal abundance of each medium. Observations of occurrences of two different breeding materials were made and recorded each day in two towns, five days per week when weather permitted. Equal periods of time were spent in locating and examining each medium for possible larval infestation. Five attempts were made daily to locate each of the two materials scheduled for collection that day in each of the two towns. If larvae were noted in the materials examined, samples were collected individually in one-fourth pint ice cream cartons equipped with friction lids and five of the most heavily infested, from the total often possible samples of each material, were brought to the laboratory. All were brought to the laboratory if fewer than five were found.

After removing the lid of the quarter-pint sample of breeding material, each such sample was placed in a one-gallon ice cream carton upon a one-inch layer of washed and sterilized moist sand to provide a place for pupation if the larvae migrated from the breeding material. The center cardboard disk in the lid of the one-gallon carton was replaced with a 24-mesh wire screen disk to permit ventilation. The rearing was done in an insectary operated at a fairly uniform temperature (mean of 81°, range 76° to 85°F.) and relative humidity (mean of 74%, range 69 to 81%). Daily checks were made on each lot at approximately the same time each day. When adults emerged, they were anesthetized with carbon dioxide and removed, then killed and stored for later identification and recording.

RESULTS

The large amount of collected data is summarized in Table 1 by the five categories of twenty breeding material items. It is quite obvious that items of animal refuse, represented by the three categories of animal pen litter, animal wastes and animal excrements, were not only the most frequently occurring but were also the most fly productive breeding materials occurring in the two study towns. All five categories were surprisingly similar in the rate of fly breeding infestation, but the less frequent occurrence of fruit and vegetable wastes and miscellaneous wastes, together with the lower fly productivity of these two categories, makes them of secondary importance.

Indoor counts of flies made during the period of this study in the same areas have shown that over 99 per cent of the flies found in occupied houses are house flies, *M. domestica*. This species' preference for human association, plus its demonstrated vector role with *Shigella* (Lindsay, Stewart and Watt, in press), needs only the wide distribution throughout the types of breeding media shown in Table 2 to establish it as the major species to be considered in this area.

A similar summary of all other common species is presented in Table 3. When compared with house fly productivity as shown in Table 2, it is apparent that no other species approached the distribution of *M. domestica* in terms of either the numbers of infestations or the per cent of media infested. The occasional high individual production of other species from any principal breeding material

TABLE 1

The occurrence, larval infestation and adult production from five principal categories of fly breeding media found in two towns in southwest Georgia, May, 1950 through April, 1951

BREEDING MATERIAL CATEGORIES	NUMBER OF OCCURRENCES	NUMBER INFESTED WITH LARVAE	PER CENT INFESTED WITH LARVAE	AVERAGE NUMBER OF ADULTS REARED PER SAMPLE
Animal pen litter*	1445	487	33.7	34.2
Animal wastes†	1060	273	25.7	60.4
Animal excrements‡	930	351	37.7	86.4
Fruit-vegetable wastes§	278	83	29.8	28.1
Miscellaneous wastes¶	478	120	25.1	17.6

* Animal pen litter refers to a mixture of domestic animal excrement, straw, hay, spilled grain and urine.

† Animal wastes included: (1) bones of cows and hogs, whether cooked or uncooked; (2) carcasses of small animals; (3) entrails found near garbage cans and poultry abattoirs; (4) fish remains; (5) paunch manure consisting of not only partially digested stomach contents of cows and hogs but their viscera and hair as well; and (6) packing plant wastes or fragments of partially processed animals ultimately used for fertilizer.

‡ The various animal excrements, including human excrement, were collections of stools or individual droppings.

§ Fruit and vegetable wastes included fruits and/or vegetables, in the field or harvested, cooked or uncooked.

¶ Miscellaneous media consisted of: (1) garbage in and around a standard container, located near an occupied dwelling; and (2) soil, which included wash and/or dishwater and worm beds.

TABLE 2

Production of Musca domestica *from five principal fly breeding media categories, 1950-51*

BREEDING MATERIAL CATEGORIES	NUMBERS OF OCCURRENCES	NUMBER INFESTED WITH *Musca domestica*	PER CENT INFESTED WITH *Musca domestica*	AVERAGE NUMBER OF *Musca domestica* PER SAMPLE
Animal pen litter	1445	260	18.0	36.2
Animal wastes	1060	102	9.6	23.2
Animal excrements	930	117	12.6	13.8
Fruit-vegetable wastes	278	38	13.7	33.4
Miscellaneous wastes	478	40	8.4	10.0

category is offset either by its relatively infrequent occurrence or by its sharp restriction to only one cateogry, as exemplified by *Sarcophagula occidua.*

Table 4 is presented to demonstrate further the exceptional adaptability of *M. domestica.* It alone was found to breed in all twenty items tested during the course of the study, a feat unequalled by any other species. This wide adaptabil-

TABLE 3

The productivity of all common fly species (other than Musca domestica*) from five principal fly breeding media categories, 1950–51*

SPECIES	BREEDING MATERIAL CATEGORIES	NUMBER OF OCCURRENCES	NUMBER OF INFESTATIONS	PER CENT INFESTED	AVERAGE NUMBER OF FLIES PER SAMPLE
Phaenicia pallescens	Animal pen litter	1445	5	0.3	1.6
	Animal wastes	1060	30	2.8	19.4
	Animal excrements	930	0	0.0	0.0
	Fruit-vegetable wastes	278	1	0.1	1.0
	Miscellaneous wastes	478	28	5.8	4.2
Phaenicia sericata	Animal pen litter	1445	4	0.2	21.5
	Animal wastes	1060	61	5.7	54.8
	Animal excrements	930	3	0.3	1.3
	Fruit-vegetable wastes	278	2	0.7	58.0
	Miscellaneous wastes	478	8	1.7	4.0
Stomoxys calcitrans	Animal pen litter	1445	120	8.3	5.1
	Animal wastes	1060	24	2.3	3.1
	Animal excrements	930	54	5.8	5.2
	Fruit-vegetable wastes	278	18	6.5	5.6
	Miscellaneous wastes	478	3	0.6	3.0
Callitroga macellaria	Animal pen litter	1445	2	0.13	28.50
	Animal wastes	1060	65	6.14	44.11
	Animal excrements	930	0	0.00	0.00
	Fruit-vegetable wastes	278	0	0.00	0.00
	Miscellaneous wastes	478	5	1.04	34.00
*Sarcophagula occidua**	Animal pen litter	1445	57	3.9	109.2
	Animal wastes	1060	15	1.0	38.1
	Animal excrements	930	118	12.7	216.7
	Fruit-vegetable wastes	278	4	1.4	194.0
	Miscellaneous wastes	478	4	0.8	6.7
Sarcophaga spp.	Animal pen litter	1445	14	0.9	8.3
	Animal wastes	1060	23	2.2	15.2
	Animal excrements	930	77	8.3	18.7
	Fruit-vegetable wastes	278	1	0.3	2.0
	Miscellaneous wastes	478	15	3.1	10.7
Muscina spp.	Animal pen litter	1445	21	1.4	3.9
	Animal wastes	1060	1	0.1	23.0
	Animal excrements	930	4	0.4	2.0
	Fruit-vegetable wastes	278	5	1.8	3.0
	Miscellaneous wastes	478	8	1.7	9.5
Hydrotaea spp.	Animal pen litter	1445	13	0.9	2.6
	Animal wastes	1060	3	0.3	1.3
	Animal excrements	930	21	2.3	9.2
	Fruit-vegetable wastes	278	1	0.3	1.0
	Miscellaneous wastes	478	1	0.2	1.0
Limnophora spp.	Animal pen litter	1445	16	1.1	1.5
	Animal wastes	1060	1	0.1	1.0
	Animal excrements	930	17	1.8	7.9
	Fruit-vegetable wastes	278	13	4.7	2.8
	Miscellaneous wastes	478	13	2.7	2.2

TABLE 3—*Continued*

SPECIES	BREEDING MATERIAL CATEGORIES	NUMBER OF OCCURRENCE	NUMBER OF INFESTATIONS	PER CENT INFESTED	AVERAGE NUMBER OF FLIES PER SAMPLE
Ophyra leucostoma	Animal pen litter	1445	15	1.0	2.5
	Animal wastes	1060	29	2.7	9.4
	Animal excrements	930	13	1.4	7.3
	Fruit-vegetable wastes	278	1	0.3	1.0
	Miscellaneous wastes	478	0	0.0	0.0
Calliphora spp.	Animal pen litter	1445	2	0.1	1.0
	Animal wastes	1060	13	1.2	50.9
	Animal excrements	930	0	0.0	0.0
	Fruit-vegetable wastes	278	0	0.0	0.0
	Miscellaneous wastes	478	5	1.0	7.0
Phormia regina	Animal pen litter	1445	3	0.2	2.3
	Animal wastes	1060	95	9.0	57.4
	Animal excrements	930	0	0.0	0.0
	Fruit-vegetable wastes	278	1	0.3	21.0
	Miscellaneous wastes	478	1	0.2	1.0

* The abnormally cold winter, beginning in November, 1950, practically eliminated *S. occidua* as a major species during the 1951 and 1952 season, as shown by studies in progress which are not reported in this paper. Since this species apparently has a narrow climate tolerance, is near its northern distribution limit and is normally not a house or human visitor, the importance of its extremely large reproductive potential, even under the favorable conditions during the 1949–50 season, is considerably decreased.

ity plus its tremendous reproduction potential probably accounts for its demonstrated ability for rapid development of insecticide-resistant populations. Items of highest protein consistency proved to be least favored and such items as paunch manure and packing plant wastes, probably because of the large amounts of partially digested stomach and intestinal contents, were found exceptionally acceptable as breeding media for this species. It is of interest to note that cow excrement and horse excrement were remarkably similar in numbers of samples and per cent of occurrences infested, as well as in the numbers of house flies produced per infested sample, in contrast to the generally accepted belief that horse excrement is highly superior to other animal excrements as a larval medium.

The seasonal distribution of seven of the most abundant species and the remainder of the common species grouped as "others" is presented in Table 5. It was found that *M. domestica* abundance was least affected by seasonal variation and, as also shown in Table 3, *S. occidua* was found to have the highest production rate of all species, but was abundant only in the hottest months. The latter species was found to be shy and timid, extremely sensitive to cold weather and, furthermore, was non-house frequenting. *Phaenicia* spp. (garbage flies), *Callitroga macellaria* and *Sarcophaga* spp. persisted in the area throughout the year and although they frequently were noted in houses they actually comprised less than one per cent of flies in houses. The other species shown in Table 3 indi-

TABLE 4

The productivity of house flies from five chief categories of breeding materials, 1950–51

BREEDING MATERIAL CATEGORIES	BREEDING MATERIAL ITEMS COMPRISING EACH CATEGORY	NUMBER OF OCCURRENCES OF ITEMS	NUMBER OF SAMPLES INFESTED WITH HOUSE FLIES	PER CENT OF OBSERVED OCCURRENCES INFESTED	AVERAGE NUMBER OF HOUSE FLIES INFESTED PER SAMPLE
Animal pen litter	Fowl	227	33	14.53	82.03
	Pig	225	51	22.66	45.51
	Cow	261	52	19.92	22.73
	Horse	209	59	28.22	29.46
	Cow & horse mixed	195	44	22.56	18.18
	Rabbit	168	9	5.35	25.67
	Goat	160	12	7.50	36.42
Animal wastes	Bones	192	1	0.52	7.00
	Carcasses	198	2	1.01	1.00
	Entrails	138	2	1.44	6.50
	Fish	168	4	2.38	2.50
	Paunch manure	188	60	31.91	27.70
	Packing plant	176	33	18.75	20.30
Animal excrements	Human	203	8	3.94	12.12
	Dog	234	20	8.54	27.10
	Cow	256	50	19.53	22.34
	Horse	237	31	13.08	21.97
Fruit - vegetable wastes	Fruit and/or vegetables, in field or harvested, cooked or uncooked	278	38	13.66	33.37
Miscellaneous wastes	Garbage	260	21	8.07	12.67
	Slop soaked soils	218	19	8.71	7.10

TABLE 5

The seasonal production of the more common flies from all items of breeding media, 1950–51

FLY SPECIES	NUMBERS REARED			
	Spring	Summer	Fall	Winter
Musca domestica	4829	6938	2488	1807
Stomoxys calcitrans	398	46	146	488
Sarcophagula occidua	4752	17251	11061	29
Phaenicia spp.	1677	1387	1342	880
Phormia regina	817	0	235	4427
Callitroga macellaria	237	791	2055	11
Sarcophaga spp.	506	1103	430	18
Others*	1038	63	122	1044
Total	14254	27579	17879	8704

* "Others" include: *Muscina* spp., *Ophyra leucostoma*, *Hydrotaea* spp., *Limnophora* spp. and *Calliphora* spp.

cated seasonal fluctuation favoring the cooler seasons and are considered of little general importance in respect to human association.

Recent work completed by Lindsay, Stewart and Watt (in press) has shown that, as a result of fly control operations in other towns in the same area in July, 1949, the *Shigella* infection rate in children in the treated towns fell and remained low as long as fly control was maintained. This rate in the untreated towns continued at a normal level during the same period. Throughout this study the authors found that all increases in fly abundance which reflected the need for additional fly control measures or the failure of measures used were the result of increases in the numbers of house flies alone. After 14 months of fly control operations, the high level of house fly resistance to DDT, chlordan and dieldrin precluded their further effective usage, yet all other common species were controlled by the residual deposits of these insecticides for several months longer.

SUMMARY

A study of production sources and seasonal abundance of the more common muscoid flies from May 1950 through April 1951 showed that *Musca domestica* was produced by a wide range of breeding media throughout the entire period, far exceeding all other fly species in this respect. This wide adaptibility, plus the house fly's enormous reproduction potential, probably accounts for its demonstrated ability to develop insecticide-resistant populations rapidly. The occasional high production of other common species from any one of five principal fly breeding media categories was offset by its relatively infrequent occurrence or its restriction to certain items of breeding material.

Animal refuse (excrements, pen litter and wastes) not only occurred most frequently of all categories of media examined, but were also the most fly productive breeding materials occurring in the two study towns. Fruit-vegetable wastes and miscellaneous wastes were found to occur less frequently and to produce fewer flies, and were considered of secondary importance.

Species composition of flies counted indoors during the period of this study showed that 99 per cent were house flies. This species preference for human association, coupled with its abundance coincidentally with diarrheal disease in the study area, further establishes it as the major species to be considered in this area. Certain other species such as *Phaenicia* spp., *Callitroga macellaria* and *Sarcophaga* spp., while frequently noted in houses, actually comprised less than one per cent of flies in houses.

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BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

The Natural History of Infectious Disease, by Sir Macfarlane Burnet, M.D., F.R.S., Director of the Walter and Eliza Hall Institute, Melbourne, Australia. 356 pp. with illustrations. New York: Cambridge University Press, 1953. $4.50.

African Highway—The Battle for Health in Central Africa, by Sir Malcolm Watson, M.D., LL.D., Hon. Consultant, Ross Institute of Tropical Hygiene, London; with an introduction by Sir Eric Macfadyen. 294 pp. with illustrations and maps. London: John Murray, 1953. Price £3/3.

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Traité de Zoologie. Anatomie, Systematique, Biologie. Tome I, Fascicule II, Protozoaires: Rhizopodes, Actinopodes, Sporozoaires, Conidosporidies. Publié sous la direction de M. Pierre-P. Grassé, Membre de l'Institut, Professeur à la Sorbonne (Paris), avec 9 collaborateurs. 1,160 pp., 833 illustrations, 2 in color. Paris (6e): Masson & Cie. 120 Boul. Saint-Germain, 1953. Price: paper 9,215 fr., boards, 9,935 fr.

Estudos sôbre o Schistosomum mansoni (1908–1916), by Prof. M.A. Pirajá da Silva. Edition published by Edgard de Cerqueira Falcão and Manuel de Sousa Varela to commemorate the 80th birthday of Professor M. A. Pirajá da Silva. Facsimiles of the original papers by Pirajá da Silva establishing the specific status of *S. mansoni*. 123 pp. with illustrations. Privately printed by E. de C. Falcão, Caixa Postal 796, Santos (S. Paulo) Brazil, 1953.

Toxic Hazards of Certain Pesticides to Man; together with a Select Bibliography on the Toxicology of Pesticides in Man and Mammals, by J. M. Barnes, B.A., M.B., Medical Consultant, Division of Environmental Sanitation, WHO, Director, Toxicology Research Unit, Medical Research Council, Gr. Britain. 129 pp., with 53 pp. bibliography. Geneva: WHO, Monograph Series No. 16. 1953. Distributed by Columbia University Press, 2960 Broadway, New York 27, N. Y. International Documents Service. Price $1.50. Fr.fr. 480, Sw. fr 6. (French edition in preparation.)

Milk Pasteurization: Planning, Plant, Operation, and Control, by H. D. Kay, C.B.E., Ph.D., D.Sc., F.R.S., Director, National Institute for Research in Dairying, Shinfield, Berkshire, England; J. R. Cuttell, A.M.I. Mech.E., Engineer, A.P.V. Co., Ltd. London, England; H. S. Hall, B.Sc., Head, Dairy Engineering Dept., National Institute for Research in Dairying, Shinfield, Berkshire, England, A. T. R. Mattick, B.Sc., Ph.D., Deputy Director, National Institute for Research in Dairying, Shinfield, Berkshire, England; and A. Rowlands, B.Sc., M.S., Managing Director, Astell Laboratory Service Co., Ltd. London, England. 204 pp., 93 Figures, 3 Tables. Geneva: WHO Monograph Series No. 14 (Published jointly by FAO and WHO and issued also as FAO Agricultural Series No. 23. 1953. (French edition in preparation.) Price $2.50, Fr.fr. 800., Sw. fr. 10.

Diphtheria and Pertussis Vaccination; Report of a Conference of Heads of Laboratories Producing Diphtheria and Pertussis Vaccines. Dubrovnik, Yugoslavia, Oct. 13–18, 1952. 88 pp. with tables. Geneva: WHO Technical Report Series No. 61, 1953. (French edition in preparation.) Price $.55, Fr. fr. 175., Sw. fr. 2.20.

Joint FAO/WHO Expert Committee on Brucellosis; Second Report. 34 pp., 1 table. Geneva: WHO, Technical Report Series No. 67, May 1953. (Issued also as FAO Agricultural Studies, No. 24). Available also in French edition. Price $.25. Fr. fr. 80, Sw. fr. 1.

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Joint ILO/WHO Committee on Occupational Health, Second Report. WHO Technical Report Series No. 66. 30 pp. Geneva: WHO. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. Price $.20, Sw.fr. 0.80.

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Atlas of Diseases—Dengue, Plague, Leprosy, Studies in Human Starvation: I. Sources of Selected Foods, II. Diets and Deficiency Diseases; Poliomyelitis; Cholera; Helminthiasis; Malaria Vectors. Compiled by the Department of Medical Geography, American Geographical Society, Broadway at 156th Street, New York 32, N. Y. 1952-1953. Price $1.25 each.

BOOK REVIEWS

Monographs in Medicine, Series 1. Ed. by WILLIAM B. BEAN, M.D. (State University of Iowa). Associate Editors: MORTON HAMBURGER, M.D. (University of Cincinnati), JOHN A. LEUTSCHER, M.D. (Stanford University) and STEWART WOLF, M.D. (University of Oklahoma). 665 pp., illustrated. Baltimore, The Williams & Wilkins Co. 1952. Price $12.00.

This volume inaugurates an important new medical monograph series, covering current significant advances in the broad field of clinical medicine, and of the applications of fundamental research to clinical problems. Series I contains contributions ranging from "Talking with the patient", by Steward Wolf, to "Chemical agents used in the treatment of inoperable and far advanced neoplastic disease" by David I. Karnofsky.

Of particular interest to workers in tropical medicine are the following articles: "Prevention of rheumatic fever" by Charles H. Rammelkamp, Jr., and Floyd W. Denny; "Amebiasis" by Henry E. Hamilton; "The present status of the chemotherapy of human malaria" by L. H. Schmidt; "The seasonal arthropod-borne, virus encephalitides" by R. Walter Schlesinger, and "Sickle cell anemia" by Byrd S. Leavell and William A. MacIlwaine.

It is emphasized that the most effective method available for the prevention of initial attacks of acute rheumatic fever is the treatment of the preceding streptococcal infection with either penicillin or aureomycin. The discussion on amebiasis deals with the clinician's responsibility, and gives a careful analysis of the recognition and diagnosis of the disease. The many drugs recommended for use in amebiasis indicate the continued unsatisfactory status of the chemotherapy of this important disease. Chloroquine and Primaquine provide mechanisms for essentially satisfactory chemotherapeutic control over all aspects of human malaria. The discussion of the virus encephalitides is particularly comprehensive. Sickle cell anemia is described as an inherited disorder apparently confined to Negroes or persons with Negro ancestry.

The volume includes important discussions of fundamental subjects such as "Physiology of the body fluids" by William W. Wallace, and "The growth and maturation of the eryth-

rocyte" by Richard W. Vilter and John F. Mueller. Interesting clinical contributions are "Precordial noises heard at a distance from the chest" by William B. Bean; "Angiocardiography" by Charles T. Dotter and Israel Steinberg; "Portal hypertension" by Mary Ann Payne and Charles G. Childs; "Pheochromocytoma" by Harry Aranow, Jr.; "Respiratory failure in neuro-muscular disorders", by Fred Plum, and "Cortisone and ACTH in infectious processes" by Max Michael, Jr. There are excellent references and a good index. The volume can be warmly recommended and it is hoped that its success in the series may be followed by subsequent volumes.

CHAUNCEY D. LEAKE

Textbook of Virology by A. J. RHODES (Research Associate, Connaught Medical Research Laboratories and Professor of Virus Infections, School of Hygiene, University of Toronto, and C. E. VAN ROOYEN, Research Member, Connaught Medical Research Laboratories and Professor of Virus Infections, School of Hygiene, University of Toronto. 2nd Edition. 561 pp. with 76 illustrations. Baltimore, Md., The Williams & Wilkins Co. 1953. Price $8.00.

This second edition is a concise treatise on the essential features of virus and rickettsial diseases of man and their causative agents. It is presented in orderly manner, contains sections on most of the essential technics and includes some very good illustrative material. The electron micrographs are especially fine.

Emphasis seems to have been misplaced in dealing with some matters. The highly specialized equipment for electron microscopy is described in considerable detail, yet the procedure of freeze-drying, so important in preparation of diagnostic reagents, vaccines, etc., is not mentioned. There are a number of misquotations in the volume and a few misstatements which might be dangerous. Example of the latter is the statement that "viruses are destroyed by the same disinfectants as destroy bacteria." Many viruses are, in fact, quite resistant to common bactericidal agents.

This volume was not intended to be a reference book and it does not deal exhaustively with any of the subjects covered. The student interested in a comprehensive report on a particular disease or its causative agent will be obliged to consult the periodical literature or other reference texts. Nevertheless, the volume supplies a good elementary coverage of the virus and rickettsial diseases of man.

KENNETH C. SMITHBURN

Die Parasitischen Würmer des Menschen in Europa, by HEINRICH BERGSTERMANN, HANS MENDHEIM, GERHARD SCHEID and JOSEF SCHMIDT. Universität München. 199 pp. 35 illus. Stuttgart, Germany. Ferdinand Enke Verlag. 1951. Price DM 21.

The book is divided into two parts. The first deals on 12 pp. briefly with the general biology, epidemiology, and pathology of helminths, and in a somewhat more detailed way with general diagnostic (15 pp.) and therapeutic (40 pp.) procedures. The second part describes those trematodes (27 pp.), cestodes (29 pp.), nematodes (54 pp.), acanthocephala (3 pp.), and gordiacea (2 pp.) that have been found as parasites of man in Europe. The more important parasites are treated according to the following scheme: Distribution, morphology, development, biology, epidemiology, pathology, diagnosis, prognosis, prophylaxis, and therapy. The book contains 35 illustrations, mainly good original photographs. It is concluded by a fairly brief bibliography and a subject index.

The book is written mainly for the physician. In view of the European post-war increase in helminthic infections, it certainly will be of value to the European clinician, especially in drawing his attention to the possibility of the occurrence of rarer parasites. While it does bring nothing new to the American professional parasitologist, the book can be recommended to students of parasitology preparing themselves for an examination in German. It is written in a very clear style which avoids almost totally the long and involved sentences so characteristic of the older German scientific literature.

THEODOR VON BRAND

A Manual of the Chiggers. The biology, classification, distribution, and importance to man of the larvae of the family Trombiculidae (Acarina) by G. W. WHARTON, Duke University, aided by H. S. FULLER, School of Public Health, Harvard University. Memoirs of the Entomological Society of Washington, No. 4. 185 pp., illustrated. Washington: Entomological Society of Washington, U. S. National Museum. 1952. Price $6.00.

This excellent manual was written with the avowed intention of bringing the pertinent information concerning chiggers between the covers of a single volume. In this the authors have succeeded admirably, although information on the Trombiculidae is accumulating rapidly as indicated by a publication noted in the appendix which adds over 70 new species to the 440 species included in the manual. The main section of the manual deals with classification of 4 recognized subfamilies, 31 genera and 17 subgenera, with keys and brief diagnoses. The species are listed with synonyms, distribution and host lists. The classification is based wholly upon larvae. A brief chapter is devoted to the impact of chiggers upon public health. This is considered under three headings: relationship to rickettsia, dermatitis and control.

A review of the literature interspersed with original observations is presented in brief chapters on the behavior of chiggers, life cycles, and anatomy, particularly of the larvae. Both external and internal anatomy are considered, with illustrations of the former. The distribution of trombiculids is considered at some length with very useful faunal and host lists. Seasonal, "diurnal" and ecological distribution are also discussed.

A rather confusing typographical error was noted in Table I which indicates a set of temperature recordings as degrees below instead of above freezing. In this same table and in Figure 2 the data on three species of chiggers are considered as comparable throughout, which seems to be incorrect. These are minor errors, however, which detract little from the value of the book.

DEANE P. FURMAN

Microbial Growth and its Inhibition. First International Symposium on Chemical Microbiology. World Health Organization, Monograph Series No. 10. Mixed language edition containing articles in English or French with summaries in both languages. 286 pp. with 67 figures. Geneva, W.H.O., Palais des Nations. 1952 Price 15/-; $3.00; Fr. fr. 960-; Sw. fr. 12-.

This monograph brings together 18 papers of a symposium held to celebrate the opening of the International Research Center for Chemical Microbiology. The monograph is a veritable gold mine of information rarely assembled in one volume. One can only indicate here the range of subjects discussed. For example, C. J. Hinshelwood deals in his paper with growth, adaptation, decline and death of bacterial populations and presents a most provocative idea suggesting that, in the intimate linkage of protein and nucleic acid, the spacings of each affect the mode of synthesis of the other; and that although the production of protein and of nucleic acid are not autocatalytic by themselves, a steady state may ensue in which the two kinds of cell material increase autocatalytically. L. Califano discusses the liberation of nucleic acid from cells under the influence of heat as an index of the heat of inactivation of the organism and of the physiological age of the cell.

D. D. Woods discusses Vitamin-B group substances in microbial metabolism and describes the methods of study and the results from each of the methods which he has obtained. J. Monod summarizes the work which he, Cohn and Torriani have conducted on the beta-galactosidase of *E. coli*, in which the synthesis by adapted cells of the enzyme protein is correlated with the disappearance of a second protein (the latter presumably the precursor of the enzyme in the non-adapted cell). S. Sokhey concludes from his studies on the lag phase in growth of *P. pestis* that this phase is one of liberation into the medium of substances essential for geometric rates of increase. E. B. Chain and others report upon the relative efficiency of different aeration mechanisms and discuss the usefulness of the vortex produced during agitation in the absence of baffles in aeration operations whereby air need not be injected through an air sparger.

A portion of this interesting volume is devoted to penicillin fermentation (M. Johnson), chemistry of terramycin, (Regna *et al.*) and the use of antibiotics as animal-growth stimulants (Weber, *et al.*) in the raising of chickens, turkeys and swine.

Two interesting papers on drug resistance (M. Welsch and L. Cavalli) are included: the latter summarizing experiments using the genetic recombination methods of Lederberg and Tatum to analyze resistance to streptomycin, azide, chloramphenicol and terramycin. B. C. J. G. Knight, in the reviewer's opinion, has synthesized a most elegant picture of the physiological background to microbial inhibition.

An excellent discussion on "lysogenesis" is given by A. Lwoff in which the author summarizes the "natural history" of bacteriophage, the kinetics of phage appearance and the data which led him to the theory that lysogenesis is an intrabacterial transmission phenomenon maintained in the absence of extrinsic phages.

L. Camici, *et al.* deal with the growth and autolysis of *Penicillium chrysogenum* in submerged culture and Sir Robert Robinson presents a paper on the structural relationships of molecules of naturally occurring organic compounds in which structural units are linked with biogenesis and biosynthesis.

SANFORD ELBERG

Epidemiology and Control of Endemic Syphilis by E. I. GRIN, M.D. WHO Monograph Series No. 11 (English edition only). 93 pp. Geneva: World Health Organization. 1953. Price 5/-, $1.00, Fr. fr. 320.–, Sw. fr. 4.–.

This is an important contribution to the field of endemic syphilis, defined by the author as a treponemal disease usually non-venereal, acquired in childhood through use of common utensils, known to be endemic in Bosnia for many centuries. This monograph gives a complete description of case finding, treatment, clinical signs and epidemiology. The author's conclusion that successful campaigns must be followed up at fairly short intervals and that all familial contacts must be treated seems vital. The experiences as related can be applied in certain areas of this country and in other parts of the world and to other treponemal diseases.

Questions by the reviewer arise in regard to the author's data given on congenital syphilis. Too high a per cent of discovered cases in all age groups in the secondary stage is indicated to make his explanation seem completely valid. It is difficult to accept so few reported cases of primary syphilis on the theory that continual, small inoculations with the treponemes sensitize the patient to the extent that secondary lesions arise without primary lesions. A third question is the explanation of sudden activation of latent syphilis to late syphilis due to superimposed infections, and the use of this as an index of incidence of the disease in the population.

This volume is stimulating, well documented and a valuable contribution in a field not heretofore well documented. It is thought-provoking and should be of great interest to all professional people interested in epidemiology of treponemal diseases.

A. FRANK BREWER

Biologisch-Medizinisches Taschenjahrbuch–1953 (Vogel). Neubearbeitet von HANS HAFERKAMP, 13th year. 508 pp. Hippokrates-Verlag Marquardt & Cie. Stuttgart, Germany, DM 7.50.

This well-printed pocket-size book is difficult to appraise by American standards. The 31 collaborators, some in respected academic positions, have created, according to the editor, a "biologic plan" of therapy which may be applied to the more common ailments of mankind—from constitutional diseases of the metabolism, skin diseases, blood dyscrasias and respiratory, digestive and renal disturbances to the maladies of infants and "supportive cure by nature" (Naturheilbehandlung) in surgery. Even a casual perusal of the text discloses a state of German medicine not reflected in current scientific journals received in this country. There is apparent a shallow uncritical acceptance of herbs, extracts, infusions and drugs long ago classed as patent medicines. Use of these preparations is encouraged by

the advertisements, book markers and other tricks of the trade so freely interspersing the leaves of the book. Over 215 pages are devoted to an alphabetical catalog and price list of the "Heil- und Kurmittel" under their respective tradenames and the address of the manufacturer. Vitamins, hormones and antimicrobial drugs receive attention and, for example, 34 different penicillin preparations and their specific usefulness are listed. As a document for a historical evaluation of the borderland of legitimate medicine it may prove valuable, but not as a guide to modern therapy.

K. F. MEYER

CORRESPONDENCE

August 27, 1953

Editor
American Journal of Tropical Medicine and Hygiene
Berkeley 4, California
Sir:

The last sentence, first paragraph, on page 570 of my article in the July 1953 issue of the Journal contains the following error:

> The statement that "the sanitary engineer whose main interest is in *progress* had better stay at home" should have been: "the sanitary engineer whose main interest is in *process* had better stay at home."

Were I writing this article today I would modify my statement on the same page that night soil is almost universally used for crop fertilization in rural Asia. Such use of night soil is the prevailing practice in some countries; in others adjoining croplands are the community defecation grounds, with resultant benefit to crops, but this is not exactly a night-soil practice.

Thank you.

Yours truly,
JOHN M. HENDERSON

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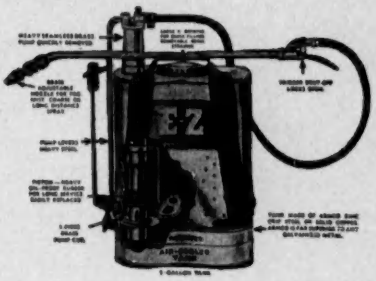

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